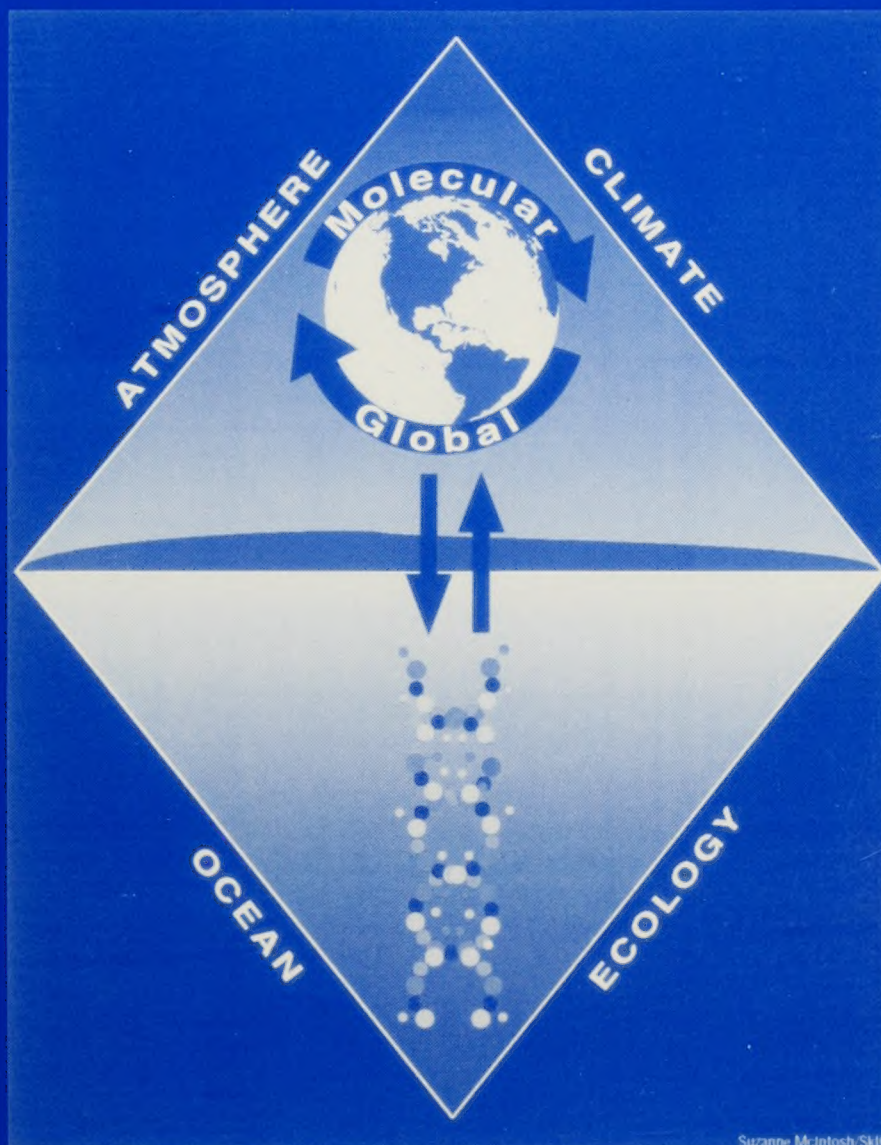


OCEAN MARGINS PROGRAM

Phase I

Research Summaries



August 1994

U.S. Department of Energy
Office of Energy Research

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Phase I

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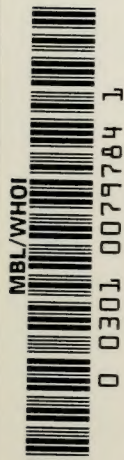
PHASE 1 PROJECTS

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RESEARCH SUMMARIES

Edited by P. Verity

June 1994



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OCEAN MARGINS PROGRAM DESCRIPTION

The Department of Energy (DOE) has traditionally supported long-term interdisciplinary studies on the structure and function of coastal ocean systems as part of its concern for sustainable development and the dispersal and fate of energy-related materials (including CO₂) in the marine environment. The approach has been to conduct regional studies along the U.S. continental shelves, utilizing moored instrumentation, ship sampling, and remote sensing to measure watermass movements; spatial and temporal concentrations of chemical species and particles; biological productivity; zooplankton grazing and bacterial respiration; ecological dynamics; and biogeochemical fluxes of organic particles, nutrients, and dissolved organic carbon between estuarine systems, the shelf, and the interior ocean.

During FY 1992, the DOE restructured its regional coastal-ocean programs into a new Ocean Margins Program (OMP), to:

Quantify the ecological and biogeochemical processes and mechanisms that affect the cycling, flux, and storage of carbon and other biogenic elements at the land/ocean interface;

Define ocean-margin sources and sinks in global biogeochemical cycles, and;

Determine whether continental shelves are quantitatively significant in removing carbon dioxide from the atmosphere and isolating it via burial in sediments or export to the interior ocean.

To achieve these objectives, the DOE has supported both process-oriented research to understand the physical, biogeochemical, plant, animal, and microbial mechanisms and interactions that affect the input, assimilation, and transformation of carbon in coastal waters and sediments; and the development of new instrumentation to obtain high frequency in-situ measurements of the environmental and biological factors affecting carbon fluxes in the ocean.

During FY 1993, the DOE launched a new molecular biology initiative within its Ocean Margins Program to provide a mechanistic understanding of the complex biological processes which mediate the carbon cycle in marine systems. Molecular biological techniques are being developed, adapted, and applied to determine how biological processes are regulated and controlled by genetic limitations and environmental variables. Research emphasis has been placed on

- (i) molecular regulation of photosynthetic carbon reduction by phytoplankton,
- (ii) molecular diagnostic markers of bacterial growth, production, and nutrient limitations to growth, and (iii) molecular techniques for elucidating metabolic pathways.

Currently, the DOE Ocean Margins Program supports more than 70 principal and co-principal investigators, spanning more than 30 academic institutions. Research funded by the Ocean Margins Program amounted to about \$6.9M in FY 1994. This document is a collection of abstracts summarizing the component projects of Phase 1 of the Ocean Margins Program. This phase included both research and technology development, and comprised projects of both two and three years duration. The attached abstracts describe the goals, methods, measurement scales, strengths and limitations, and status of each project, and level of support. Keywords are provided to index the various projects. The names, addresses, affiliations, and major areas of expertise of the investigators are provided in appendices.

Planned Activities

During the past two years, OMP scientists have developed an integrated multidisciplinary science plan to quantify the physical and biogeochemical processes affecting carbon fluxes, nutrient cycles, and ecological dynamics along the ocean's margins. Although this plan is generic in nature, it forms the scientific framework for melding the research summarized in this document into a field experimental program to assess the exchange of carbon and other biogenic elements between estuarine systems, the shelf, and the interior ocean. This field experimental program will be conducted in the coastal waters near Cape Hatteras, North Carolina, where carbon burial in sediments and carbon export (as either DOC or POC) into the interior ocean, are expected to be maximum.

Program Schedule

- FY 1995: Conduct an outside peer review of physical, biogeochemical, and biological research projects and award new competitive research grants to address the field experimental needs of OMP
- FY 1995: Initiate the field experimental phase of OMP at Cape Hatteras
- FY 1996: Conduct an outside peer review of molecular biological research projects and coordinate this mechanistic research with OMP field activities
- FY 1996: Make fully operational the field experimental phase of OMP
- FY 1997: Evaluate OMP's field and laboratory measurements and assess the role of the coastal ocean in the global flux of carbon
- FY 1997: Begin using OMP results to: (i) improve ocean-circulation, ocean-atmosphere-interaction, global-change, and global-carbon-cycle models, (ii) develop remote sensing algorithms for productivity in coastal areas, and (iii) plan the next phase of the OMP.

FY 1998: Initiate the next phase of OMP by identifying a new experimental location to confirm the representativeness of the Cape Hatteras results, or by addressing new policy-relevant issues in coastal science.

Program Interfaces

The DOE Ocean Margins Program is fully integrated with the National Science and Technology Council's (NSTC) Committee on Environment and Natural Resources (CENR), as a focussed effort within the Subcommittee on Global Change Research and as a contributory effort within the Subcommittee on Water Resources and Coastal and Marine Environments. The OMP is the major U.S. integrated multidisciplinary research effort for understanding the ocean margin's role in the global carbon cycle. It is strongly linked with the JGOFS and GLOBEC Programs because there is compelling evidence that the input of nutrients to coastal areas from land-based sources (via rivers and the atmosphere) and from interior-ocean sources (via coastal upwelling and frontal exchange), cause as much as 30%-50% of the total primary production of the global ocean to occur along its margins. The OMP and its scientific researchers are also interacting with IGBP's LOICZ Program and several U.S. Agency programs concerned with quantifying the processes that affect the transport and fate of water, carbon, nutrients, biota, sediments, and pollutants in changing coastal environments, including EPA, MMS, NASA, NOAA, ONR, and NSF's Program on Coastal Ocean Processes.

Policy Payoffs

Research within the OMP is important for; (i) predicting the dispersal and biogeochemical fate of carbon, nutrients, and other biogenic elements in coastal waters, (ii) quantifying primary productivity and ecological dynamics (structure and function) in ocean-margin systems, and (iii) examining the impacts of nutrient loading and other pollutants from anthropogenic sources.

Quantitative information on the flux and fate of CO₂ and biogenic elements at the land/ocean interface is important for the IPCC and other integrated assessments of sources and sinks in the global carbon cycle. In addition, quantitative information on coastal processes underpins policy decisions on resource management in changing coastal areas.

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PROJECT TITLE

THE SIZE DISTRIBUTION AND CONCENTRATION OF
SEDIMENTS NEAR THE SEABED: A SPECIFICATION OF
THE BOTTOM BOUNDARY CONDITION

AMOUNT OF FUNDING FY 1994: \$84 K

SUMMARY OF GOALS

To the extent that organics are sequestered or derived from bottom sediments, it is important to determine this flux. Our interest is in the estimation of sediment related input/output at the seafloor. The sediments, stirred by the wave-induced near-bed oscillatory motion, produce a strong nepheloid layer of the order of 5-10 cm thick in a region termed the 'wave boundary layer'. This layer contains all that material which is either of high density, large, or both so that the net result is that it has a high settling rate. Significant mass transport of such large material can occur in the wave boundary layer. To date, no precise measurements of the sediment have been made; single parameter data such as optical backscatter are subject to enormous errors due to a spatially and temporally varying size distribution - to which these sensors are highly sensitive. Our approach employs a new sensor system, developed as a follow up of one used on the California shelf in the experiment STRESS. The instrument is based on laser diffraction by a particle ensemble. The angular scattering pattern is recorded and subsequently mathematically inverted to obtain size spectra and concentration.

The present effort involves development of a sensor which can make these measurements close to bed, unlike its predecessor used in STRESS. We have used fiber-optics to create a remote sensor head. The instrument is called LISST-3.

Special goals include:

- (1) Development and test of the new instrument;
- (2) Field deployment from a cruise in July, 1994;
- (3) Analysis of data recovered in August;
- (4) Interpretation of carbon content with the fluorescence data of Dr. Steve Lohrenz of University of S. Mississippi.

- (5) Combining the bottom stress estimates from BASS (data of Dr. William and Churchill of WHOI), to reach a formulation for the bottom boundary condition for sediments, relating stress and a reference concentration.

SPATIAL AND TEMPORAL SAMPLING SCALES

The LISST instrument measures the size distribution and concentration of particles at a minimum of 7.7 cm above bed - a distance chosen to ensure that the instrument does not cause local scour. The sample volume dimensions are 5 mm long x 3 mm dia. Samples are possible at 0.1 second intervals, however such sampling requires enormous memory. As a result our present sampling scheme is to obtain 1Hz data for 100 seconds, every 6 hours. An on-board pressure sensor, recording synchronously, permits correlation of particle data with sediments.

METHODS AND PLATFORMS

The instrument is mounted on Dr. Williams' tall tripod.

STRENGTHS AND LIMITATIONS OF PROPOSED RESEARCH

The measurement is new and sorely needed for boundary layer physics. A limitation is that the measurements do not specifically separate organic mass from total sediment mass. This will be addressed by combining Dr. Lohrenz's fluorometry data.

STATUS OF RESEARCH

The LISST-3 instrument was deployed on the mid-Atlantic bight July 23. Recovery cruise is planned for late august. Data analysis and subsequent cruise participation will then follow.

Keywords: benthic boundary layer, particle size spectra, suspended sediments

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PROJECT TITLE

MULTIPLE TRACER MEASUREMENT OF THE COUPLING
BETWEEN BENTHIC CARBON FLUXES AND
BIOTURBATION ACTIVITY DURING THE "SPRING
BLOOM"

AMOUNT OF FUNDING FY 1994: \$97 K

SUMMARY OF GOALS

Our primary goals are to quantify and model: 1) the net fluxes of reactive biogenic debris (planktonic C_{org} , SiO_2 , $CaCO_3$) to the seafloor, 2) the resulting net sedimentary remineralization rates (N, ΣCO_2 , Mn), and 3) associated benthic community activities which partially control remineralization, including bioturbation, benthic shell deposition, and microbial abundance/biomass production. The emphasis is on fluxes of sedimentary material and processes with characteristic reaction or transport timescales of several weeks to months, corresponding to and integrating over major nonsteady-state seasonal water column events such as plankton blooms or storms. These timescales lie between those typically addressed in high resolution water column studies (hrs,days) and the longer term burial fluxes elucidated by deeper sediment properties (yrs, Kyr). Methodologies (see below) are centered on *solid phase* reactive or natural tracer constituents in surface sediments, such as chloropigments and ^{234}Th , for which decomposition kinetics are either known or can be relatively accurately inferred, and which allow model conversion of measured sedimentary inventories into mass flux estimates. The methods employed are particularly amenable to functional spatial mapping and thus are useful in complex regions where variability requires relatively dense sampling to accurately resolve patterns. The primary measurements proposed are an appropriate adjunct to direct measurements of solute fluxes such as benthic O_2 , ΣCO_2 , and nutrient fluxes, to made primarily by other investigators.

SPATIAL AND TEMPORAL SAMPLING SCALES

Spatial sampling scales and distribution are yet to be determined (see Status of Research) but 20 - 25 stations per cruise (~10 d), depending on the exact constellation of measurements, represents present capacity. Determination of station sites will be made in conjunction with other investigators based on total program goals. Because substantial benthic delivery of biogenic

debris likely occurs during a spring bloom period, we envision at least two seasonal cruises corresponding to pre-bloom and early post-bloom, with an additional sampling during an intermediate non-bloom interval.

METHODS AND PLATFORMS

Remotely obtained sediment box cores and bottom water samples (SPM, Chl-a/ C_{org} ratio) represent the primary collection methods. Conditional topographic sampling using specifically oriented samples obtained by submersibles may be used where possible in conjunction with other investigators such as R. Jahnke and L. Benninger. Our emphasis will be on properties that can potentially provide estimates of reaction rates and net fluxes, lend themselves to mapping over large regions, and which can be related to planned water column measurements such as microbial metabolic activity and suspended matter pigments. We propose to: 1) measure ^{234}Th , (^7Be will be attempted) Chl-a, and biogenic SiO_2 on submersible and surface retrieved cores and bottom water suspended matter. The resulting profiles will be used to estimate particle reworking rates near the sediment-water interface, derive *in situ* rate constants for Chl-a and SiO_2 decomposition, and model the likely reactive biogenic debris flux to the bottom for comparison to solute-based estimates made predominantly by R. Jahnke. 2) We will take X- radiographs of sedimentary structures in cores to evaluate likely modes and relative dominance of bioturbation or physical transport processes at each site. 3) We will quantify both live and death assemblages of shell-bearing benthos (molluscs, forams) and examine specific components of debris for indicators of benthic deposition or dissolution of CaCO_3 . Patterns of live:dead:total abundances in principle allow estimates of net dissolution or precipitation of benthic carbonate between sites and will be factored into interpretation of CO_2/O_2 flux balances. Comparison of live : dead carbonate bearing infauna also provides insights into biological controls on mortality (i.e., predation) as well as indicating lateral transport processes of biogenic debris. 4) Bacterial abundances will be assessed by direct count techniques using epifluorescence microscopy and acridine orange stained samples. Community average and dominate bacterial species-specific growth rates will be evaluated by measuring rRNA content of individual cells and rRNA frequency of the total population. P. Kemp's group at Brookhaven National Laboratory are developing rRNA specific oligonucleotide probes to a subset of numerically and biomass dominant bacteria in coastal sediments. We will work with them in using these probes to evaluate the population dynamics of dominant metabolic sediment microbes in the study area and relate them to environmental properties such as Corg flux. 5) We will attempt to measure net remineralization rates in 0-5 cm surface sediment (ΣCO_2 , NH_4^+) using a whole core incubation technique (Aller and Mackin, 1989; Aller, et al., 1994) on retrieved cores at shallower water sites and *in situ* at any submersible study stations. 6) In selected cases (i.e. muddy sediments, < 200 m depth), biogenic irrigation will be measured using Br^- tracer methods on shipboard incubated cores, in order to further constrain benthic community influence on remineralization. These cores could be used to estimate benthic O_2 fluxes as required, but in general we do not plan extensive direct solute flux measurements or detailed pore water profile work-ups.

STRENGTHS AND LIMITATIONS OF PROPOSED RESEARCH

The measurements chosen in principle allow inference of benthic fluxes of reactive debris (e.g., plankton C_{org} , SiO_2 , $CaCO_3$) over monthly timescales, remineralization rates and reaction pathways internal to the sedimentary deposit (N, Mn), and relative importance of physical or biological control of transport processes (^{234}Th , x-radiographs, Br^- , live/dead shell analysis) at a given site. The comparison of live/dead carbonate bearing infauna also provides insights into biological controls on mortality (i.e., predation) as well as indicating transport processes such as lateral input of debris. A major advantage of using sediments for this purpose is that they provide integrated measures of net processes operating in an area, direct measurements of boundary conditions associated with net burial of biogenic debris, and are less subject to the high frequency and spatial variations typical of water column measurements while still providing insight into nonsteady state processes. A substantial uncertainty in the present case is that the chlorophyll-based models of C_{org} flux have been derived exclusively from ongoing research in Long Island Sound and may not be as successful in the Hatteras field region. The sediment incubation reaction rate techniques are also best done *in situ* at depths > 200 - 500 m. Applicability of the proposed methods will be examined during preliminary study in August 1994.

STATUS OF RESEARCH

Research to date has been based in Long Island Sound and has shown that the techniques outlined above provide a coherent, quantitative depiction of material flux and transport processes in bottom sediments during a spring bloom period. Initial extension of the approaches and evaluation of their use in the Hatteras field region will be done during late August 1994 in conjunction with R. Jahnke and L. Benninger. A submersible will be employed to obtain samples within characteristically complex terrain in the study area. Results of the measurement suites outlined previously will be compared with solute and burial fluxes determined by Jahnke and Benninger, and act as a basis for future effort or modification thereof.

Keywords: Biogenic fluxes, sedimentary remineralization, benthic communities

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PROJECT TITLE

TRANSFORMATION RATES AND FATE OF DISSOLVED,
COLLOIDAL, AND PARTICULATE FORMS OF ORGANIC
CARBON IN OCEAN MARGINS

AMOUNT OF FUNDING FY 1994: \$89 K

SUMMARY OF GOALS

The research that our group is conducting as part of the Ocean Margins Program is intended to directly ascertain the flux of dissolved, colloidal and particulate organic carbon along and across the Atlantic continental margin. Through the use of natural isotopic tracers, the extent and rates of transformation between each of these pools will also lead to a better understanding of the interactions of organic carbon in its different physical states. Our main goals are: 1) to differentiate the apparent radiocarbon age(s) of dissolved, colloidal and particulate organic carbon (DOC, COC and POC) on different regions of the continental shelf and compare these ages to those for the open North Atlantic Ocean (Sargasso Sea); 2) to identify the sources of organic carbon to continental shelf waters; 3) to estimate the fluxes of organic carbon along and across the continental shelf to the open ocean; and 4) on the basis of simultaneous measurement of carbon and thorium isotopes in several fractions of the DOC and POC, determine the residence times and transformation rates of carbon between each of the pools.

SPATIAL AND TEMPORAL SAMPLING SCALES

The spatial sampling scale for our program is the entire western Atlantic continental shelf between George's Bank and Cape Hatteras. To date, our coverage has consisted of three transects across the shelf: one south of Long Island, one between Chesapeake and Delaware Bays and one at Cape Hatteras. Depth profiles are conducted at three stations on each transect, the

deepest to 1000 m depth at the seaward-most station on each transect. We anticipate that our temporal coverage of this region will be on a seasonal basis during the upcoming field year(s).

METHODS AND PLATFORMS

Our methods focus on the measurement of total organic and inorganic carbon pools and the carbon isotopic composition (^{14}C and ^{13}C) of each pool. Carbon contents of each of the pools are determined by high-temperature combustion (DOC, COC and POC), ultra-violet oxidation (DOC, COC) or stripping after acidification (total dissolved inorganic carbon, DIC), followed by purification and quantification on vacuum extraction lines. Isotopic measurements of carbon in each of the pools are made by stable isotope ratio mass spectrometry (^{13}C) and accelerator mass spectrometry (^{14}C). Thorium isotope measurements of selected samples are also being made on in situ pump samples taken in conjunction with K. Buessler. Appropriate sampling platforms include vessels with standard hydrowire capabilities and the ability to accommodate a clean van for natural radiocarbon work.

STRENGTHS AND LIMITATIONS OF PROPOSED RESEARCH

The strength of this approach of measuring carbon distributions and fluxes is that information is obtained on both the sources and residence times of carbon in each of the organic and inorganic carbon pools. Coupled with hydrographic and water mass circulation data, this information is critical for determining the net fluxes of each of the bulk carbon pools along and across the shelf and away from their indicated source areas. Thus, average rates and residence times of the carbon pools are obtained. This information then serves as a benchmark from which to interpret the distributions and fluxes of all other components of the DOC, COC and POC pools.

STATUS OF RESEARCH

Prior to our first cruise to the Atlantic continental margin, work on the distribution and fluxes of organic carbon and isotopes in DOC, COC and POC pools of the eastern North Pacific identified sampling and analytical challenges that were resolved prior to sampling on the Atlantic shelf. Data from our North Pacific site serves as a starting point from which we may be able to anticipate the various relationships between each of these three pools on the Atlantic shelf. Samples were collected during a 2-week cruise to the Atlantic shelf in April 1994 and are currently being processed for isotopic characterization.

Keywords: DOC/POC/COC fluxes, sources, natural isotopes, residence times

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PROJECT TITLE

MECHANISMS OF DISSOLVED ORGANIC CARBON
CYCLING IN AN OCEAN MARGIN

AMOUNT OF FUNDING FY 1994: \$101 K

SUMMARY OF GOALS

Dissolved organic carbon (DOC) is the largest organic carbon reservoir in the ocean. It is important to understand the processes by which DOC is cycled because changes in the oceanic DOC pool could affect atmospheric carbon dioxide concentrations. Phytoplankton are the dominant source of marine DOC, but the mechanisms and pathways of DOC formation are poorly understood. This study will focus on the production of DOC during herbivorous grazing on both eucaryotic and procaryotic phytoplankton. Herbivory is likely the major pathway for the transformation of organic matter from particulate to dissolved form by grazers. A combination of laboratory and field experiments will be used to determine the following:

- the relative importance of herbivory for DOC production,
- the rates and chemical characteristics of DOC production by natural communities and particular species of phytoplankton, micro- and mesozooplankton,
- the effect of phyto- and zooplankton species on the quantity, molecular size, and composition of released DOC,
- the degree of coupling between DOC production and bacterial utilization.

SPATIAL AND TEMPORAL SAMPLING SCALES

We propose characterizing herbivorous grazing and DOC release at inner, mid and outer shelf locations within the framework of process-oriented "transformation cruises." The

experimental timescales of the grazing experiments are well-matched with the free-floating sediment trap deployments of ~2 days. These experiments should be conducted within the vicinity of the cluster mooring sites. These processes will be measured during seasonal cruises to the study region off Cape Hatteras.

METHODS AND PLATFORMS

Laboratory experiments will be conducted in mesocosms with phytoplankton and zooplankton species representative of the major taxa known to play a major role in coastal marine systems. Grazing by microzooplankton and concomitant DOC production will be estimated using the dilution technique of Landry and Hassett (1982). Bacteria will be DAPI-stained and enumerated using epifluorescence microscopy (Porter and Feig, 1980), and phytoplankton and protozoa will be counted using either epifluorescence or inverted microscopy (Strom and Buskey, 1993). Bacterial production will be estimated from rates of protein synthesis as measured by the incorporation of ³H-leucine (Kirchman et al., 1985). Tangential-flow ultrafiltration will be used for separating DOC into various molecular sizes for chemical characterization (Benner, 1991). Chemical characterizations of samples will include measurements of DOC by high-temperature combustion (Benner and Strom, 1993), dissolved carbohydrates by spectrophotometry (Pakulski and Benner, 1992), neutral sugars by HPLC (Mopper et al., 1992), dissolved amines by HPLC (Gardner and St. John, 1991), and pigments by HPLC (Wright et al., 1991).

STRENGTHS AND LIMITATIONS OF PROPOSED RESEARCH

The proposed experiments will help elucidate the fundamental mechanisms and major pathways of carbon flow between the autotrophic and heterotrophic components of marine food webs. Knowledge of mechanisms and pathways of carbon flow is essential for determining environmental factors that regulate the ultimate fate of carbon in the ocean. The proposed grazing experiments require 1 to 2 days to complete so spatial coverage during cruises will be limited and we must assume that the measured rates of DOC production and consumption are representative for the general study region.

STATUS OF RESEARCH

This is a new project that will start on September 1, 1994.

Keywords: DOC, zooplankton, grazing, phytoplankton

PRINCIPAL**INVESTIGATOR(S)**

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PROJECT TITLE

CARBON CYCLING ON THE CONTINENTAL MARGIN:
EVIDENCE FROM SEDIMENT 14-C AND NUTRIENT
ELEMENTS

AMOUNT OF FUNDING FY 1994: \$119 K

SUMMARY OF GOALS

- a. Identification of sites of deposition of modern organic carbon from estuaries to the continental rise. Our tracer for modern carbon is bomb 14-C, manifest as either future, or anomalously young, age in surface sediments. We will use fallout radionuclides (Pu; 137-Cs if present) and natural 210-Pb as indices of modern particle accumulation or mixing.
- b. Modern rates of carbon burial. In environments of rapid modern sediment accumulation (probably > ~3 mm/year) fallout nuclides and 210-Pb will be used to estimate rates of sediment accumulation. Together with carbon concentrations, the accumulation rates will yield carbon burial rates. Since burial of carbonate-C and organic-C affect atmospheric CO₂ in opposite senses, we will quantify both carbonate carbon and organic carbon.
- c. Nutrient-to-organic-carbon ratios in modern sediments. Nitrogen and phosphorus will be determined in modern sediments so that we can quantify nutrient/carbon ratios in deposition and burial. If organic carbon burial on the continental margin is to significantly affect atmospheric CO₂, nutrients must escape burial through preferential recycling.
- d. 14-C signal in modern organic production. Analysis of plankton and Sargassum samples will directly characterize the carbon-isotopic signature of present-day primary production. When available in sufficient quantity for analysis, benthic macroinvertebrates will reflect the carbon-isotopic composition of organics reaching the benthic food web.
- e. Long-term trends in carbon and nutrient burial. Carbon, carbon isotopes, and nutrients in long cores (≥ 1 m) will be used to quantify burial rates over hundreds - thousands of years. Deeper profiles will also aid in interpretation of near- interface, modern phenomena of deposition and burial.

SPATIAL AND TEMPORAL SAMPLING SCALES

a. Shallow-water sediments. Sediments of the shelf and upper slope probably experience seasonality in temperature, light penetration, and flux of organic carbon. To test for temporary storage of newly produced organic matter in these sediments, we will need to contrast samples collected in productive and non-productive seasons.

Spatially, our preliminary data suggest trends over latitude, with sediments becoming finer and more carbon-rich from north to south within the field area. We need additional samples to better define gradients over latitude, probably emphasizing depths > 75 m.

b. Deep-water sediments (depth > ~400 m). Temporal distribution of sampling should be less critical for these water depths, although newly-produced organic matter is probably more likely to be detected or intercepted in transit during the first few months after major blooms. Spatial variability must be assessed on small and large scales. Extreme bottom roughness, generally visible as downslope ridges and valleys, must influence transport and deposition of organic matter. We need closely-spaced samples, with precise control over sample location, to understand the effects of this topography; some of this sampling may require use of submersibles. On a larger scale, we need to test for intensification of sediment transport and accumulation southward toward Cape Hatteras; a very high accumulation rate has been reported for one site near Cape Hatteras, but there are too few data in the field area to place this number in perspective. For depths exceeding 1000 m, there are very few data on sediment properties or accumulation rates; because seaward transport could result in deposition of organic matter on the lower slope or upper rise, we shall need to do at least survey sampling in this province.

METHODS AND PLATFORMS

We collect most of our sediment samples from surface ships, using grab- or core-samplers. Most ships are capable of handling this equipment. However, while we have our own sediment grabs and a device which collects short (≤ 25 cm) cores, we rely upon ships to provide equipment for collecting longer cores (box cores, gravity cores, piston cores, Kasten cores, etc). Ships which do not routinely sample sediments may be poorly equipped to do so; for example, in May 1993 box-coring from the *Gyre* was usually unsuccessful.

Because of the time involved in subsampling sediment cores, sediment sampling in the OMP field area (short transits between stations) is advantageously combined with other ship uses. Since we are collecting samples for measurement of natural levels of ^{14}C , however, it is desirable that we not share cruise time with investigators who perform ^{14}C tracer experiments at sea.

STRENGTHS AND LIMITATIONS OF PROPOSED RESEARCH

Strengths. Sediments are the natural integrators of carbon-cycle processes in the sea, providing time-averaged information on particle transport and deposition which is free from assumptions about the behavior of artificial particle collectors. Thus sediment chronologies are the natural (and perhaps the only reliable) techniques for assessment of the long-term (years - millennia) rates of carbon burial and its impact on atmospheric CO₂.

Limitations. Along most of the Atlantic margin of North America long-term sediment accumulation rates do not exceed a few tens of centimeters per 1000 years. Such rates provide little resolution of modern trends in sediment accumulation. Indeed, in many places bioturbation has mixed the sedimentary record of the last two centuries, the period of most critical human impact on the carbon cycle, into sediments deposited during the last 1000- 2000 years. Thus inferences from sediments as to the recent past are often more model-dependent than one would like. In addition, sedimentary geochemistry and radiochemistry are labor-intensive, and very little of the labor can be performed at sea.

STATUS OF RESEARCH

Relative to our expectations, we are currently sample-rich and data-poor; this resulted from our spending a month at sea in year two, rather than the five days for which we had planned. We have made good progress on screening analyses of our several hundred samples: we have loss-on-ignition for all, gamma spectrometry on most, scattered grain-size measurements (emphasizing % mud). We are presently implementing carbonate analysis, and as we will use the same samples for multiple measurements, this will carry through to most of the remaining wet-chemical and radiochemical analyses. The aforementioned analyses we regard as "screening" for 14-C, in that the data are needed either to assess the feasibility of 14-C determination (LOI or % organic C, % carbonate C) or to place 14-C data in context.

Our samples for 14-C analysis are stored frozen from the time of collection. Advice of colleagues that these samples should be freeze-dried, rather than oven-dried, for 14-C analysis has been borne out in preliminary comparisons of carbon contents in sample splits dried by both methods. Lacking access to a conventional freeze-drying apparatus, we have assembled one from parts, and we are systematically freeze-drying samples for 14-C analysis. Our large samples require 1-2 days to freeze-dry. Dried samples are again stored frozen.

Planning for, executing, and curating and screening samples from, our cruises has left too little time in the laboratory, and the 14-C line is still not operational. We plan final modifications of the combustion train for early this summer. Then we will begin synthesizing and counting benzene. Again, however, we are committed to substantial field work for which we did not plan: the *Gyre* cruise in June-July (16 days) affords an opportunity to sample Chesapeake Bay; a NOAA/DOE-funded submersible in August provides access to the seafloor,

thus a chance to sample the rough bottom topography *in situ*. We consider our participation in these cruises to be important, but it comes at a price of further delay in our laboratory work.

Keywords: carbon burial, deposition sites, sediment nutrient ratios, isotopes

**PRINCIPAL
INVESTIGATOR(S)**

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PROJECT TITLE

THE DYNAMICS OF CARBON EXCHANGE IN
VERTICALLY STRATIFIED COASTAL
BACTERIOPLANKTON COMMUNITIES

AMOUNT OF FUNDING FY 1994: \$86 K

SUMMARY OF GOALS

My immediate goal is to complete the analysis of batch samples obtained from the March '94 and upcoming June '94 Cape Hatteras cruises for bacterioplankton growth-state. Data sets will include: 1) measurements of DnaK protein as an indicator of growth state and therefore nutrient (e.g. carbon) availability; 2) total protein levels; and 3) gram negative-specific protein levels. Protein levels will be used as general measures of productivity and for normalization of other data. These data sets will be integrated with those of Dr. S. Giovannoni's group (OSU) involving bulk rRNA/rDNA levels in replicate sample sets.

SPATIAL AND TEMPORAL SAMPLING SCALES

Spatial and temporal sampling scales will assess bacterioplankton growth state on multiple transects and depths at the Hatteras site during sampling periods which are expected to vary in the degree of stratification of the water column. During the March '94 Hatteras cruise, samples were collected at four stations from each of three transects (surface samples), and at an outer station at 6 depths. During the June '94 Hatteras cruise, we intend to concentrate on depth profiles at both inner and outer stations.

METHODS AND PLATFORMS

Water samples containing bacterioplankton are collected by rapid filtration and the resulting filters are sealed in plastic and frozen at -200 °C. All samples are currently obtained from shipboard collections.

STRENGTHS AND LIMITATIONS OF PROPOSED RESEARCH

The expected data will directly assess the proportion of growing versus nongrowing gram negative bacterioplankton in the Hatteras collection site during the two sampling periods e.g. March and June '94. In conjunction with Dr. Giovannoni's data analysis on rRNA/rDNA we should also be able to comment on the most important taxa contributing to changing biomass.

STATUS OF RESEARCH

Pilot batch sample analysis has been completed using Pacific (OR) coastal water samples. DnaK protein was successfully detected in these samples and quantitated by transmittance densitometry of Western blots probed with an anti-DnaK monoclonal antibody. These data were normalized to total protein to assess the growth state of the bulk cell population. In addition, Hatteras samples have been collected (March '94 cruise) and are currently undergoing analysis.

Extension of these methods will focus on the analysis of individual cells as a means of refining "bulk" sample data. Significant effort is being devoted towards the development of single cell analytical techniques for DnaK quantitation which will be crucial for determining the contribution of individual dominant bacterioplankton taxa to carbon respiration.

Keywords: microbial, growth rates, DNAK, protein

**PRINCIPAL
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PROJECT TITLE

TRANSFORMATION RATES AND FATE OF DISSOLVED,
COLLOIDAL AND PARTICULATE FORMS OF ORGANIC
CARBON IN OCEAN MARGINS

AMOUNT OF FUNDING FY 1994: \$228 K

SUMMARY OF GOALS

The goal of this work is to use natural isotopic tracers (^{234}Th , ^{228}Th , ^{230}Th , ^{12}C , ^{13}C , ^{14}C) measured on different organic carbon phases to provide information on turnover rates, sources and fate of organic carbon in ocean margins.

SPATIAL AND TEMPORAL SAMPLING SCALES

Sampling scales involve both an examination of seasonal signals in the DOC/COC/POC pools (i.e. covering a range of biological settings and physical conditions), and spatial contrast with onshore/off shore changes in isotopic characteristics, and profiles from surface to depth over the slope.

METHODS AND PLATFORMS

The work needs to be conducted from research vessels, perhaps focusing on transects from the shelf to the slope at a limited number of stations ($n=3$) and depths ($n=5$ max. over slope). Water can either be collected with bottles and processed via cross-flow filtration procedures on board, or using in-situ CFF pumps now under development for OMP.

STRENGTHS AND LIMITATIONS OF PROPOSED RESEARCH

The strengths of this work include the ability to determine turnover rates of DOC/COC/POC via thorium isotopes, as well as the ability to identify carbon sources and "ages" using carbon isotopes. Weakness include the need to operationally separate colloids and particles matter from seawater for chemical analysis, so our results are specific to the classes of compounds separated by these techniques. There is also a considerable investment in people power (and \$) which is required for a single point in space and time, hence the spatial and temporal coverage will be limited.

STATUS OF RESEARCH

We have just completed our first cruise where all of the above mention tracers will be measured on the same samples (April cruise from George's Bank to Hatteras). Since most of the analyses are time consuming, we will not have many results until this fall. Independent work by the PI's (Buesseler, Moran, Bauer, Druffel) suggests that the results will provide the first look at colloid turnover rates and fate in a systematic survey of the US margin region. This new data will prove quite valuable in planning the scope of future Hatteras work, as this field is still in its infancy.

Keywords: isotopes, carbon sources, turnover rates, fluxes

**PRINCIPAL
INVESTIGATOR(S)**

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PROJECT TITLE

DETERMINATION OF PARTICULATE ORGANIC CARBON
FLUXES USING THORIUM-234

AMOUNT OF FUNDING

FY 1994: \$0 K (This project is not currently funded within
OMP)

SUMMARY OF GOALS

The goal is to quantify the vertical flux of particulate organic carbon from the surface ocean.

SPATIAL AND TEMPORAL SAMPLING SCALES

The vertical particle flux will vary with season and location on the same scale as the biology and particle resuspension fluxes. Since ^{234}Th has a 24 day half-life, its distribution at any one time will reflect the net effect of scavenging and particle export over a period of a couple of months. To make a reasonable ^{234}Th budget off of Hatteras, one would hope to have sampling opportunities every other month, perhaps on a spatial scale equivalent to the 24 stations along 2-3 off shelf transects and one along shelf line identified in previous OMP documents.

METHODS AND PLATFORMS

Thorium can most efficiently be sampled using *in-situ* pumps. In JGOFS, we reduced the sample load considerably combining water from multiple depths into a "vertically integrated" average ^{234}Th activity, for example from the 0-100m layer. One could also sample from an underway surface pump for mixed-layer ^{234}Th activities. A separation into dissolved and particulate phases is made with in-line filtration, and POC/PON are determined as well.

STRENGTHS AND LIMITATIONS OF PROPOSED RESEARCH

Strengths include the ability to quantify particle export fluxes on spatial and temporal scales equivalent to the biology and physics of the region. The measurement is relatively easy,

and much of the work can be completed at sea by a single person. Limitations include the need to rely on the empirical ratio of $\text{POC}/^{234}\text{Th}$ to convert from ^{234}Th flux to POC flux. Also, this budgeting process will not work if the ^{234}Th inventory and particles samples are severely impacted by resuspension. One would need to work in deeper waters ($z > 100\text{m}$) and/or in stratified surface waters which are isolated from direct bottom effects. As such, one would determine the next export of POC out of the upper mixed layer only.

STATUS OF RESEARCH

This research has been ongoing since the JGOFS pilot programs in 1988. Sea-going sampling and analytical equipment exists, as well as models for interpreting the results. This type of work is not currently funded as part of the OMP.

Keywords: vertical fluxes, thorium, POC

**PRINCIPAL
INVESTIGATOR(S)**

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PROJECT TITLE

ASSESSING INORGANIC NITROGEN AND PHOSPHORUS
UTILIZATION BY BACTERIA USING AN
IMMUNOFLUORESCENT METHOD

AMOUNT OF FUNDING FY 1994: \$108 K

SUMMARY OF GOALS

The goal of my program is to assess the utilization of inorganic nutrients (NH_4^+ , NO_3^- , PO_4^{3-}) by bacteria in planktonic ecosystems within the context of OMP. Controversy has arisen in recent years on the role of bacteria as nutrient remineralizers in aquatic ecosystems. The traditional role of bacteria as sources of inorganic nitrogen and phosphorus (via the decomposition of organic matter) has been challenged by the recognition that N and P may not be present in sufficient quantities in the organic compounds used by bacteria for growth. My goal is to develop and apply immunological methods that can be used to assess the physiological status of bacteria and the conditions that promote this behavior.

SPATIAL AND TEMPORAL SAMPLING SCALES

Ideally this program will examine the physiological status of bacteria along seasonal, geographical and vertical transects to determine the extent to which bacterial uptake of inorganic N or P exists. My hypothesis is that bacterial utilization of inorganic N and P is promoted by conditions of oligotrophy and is related strongly to the nutritional status of the algae in a particular environment (more severely nutrient-limited algae result in conditions in which the bacteria are also nutrient limited. Therefore, greater utilization would be expected in summer versus winter, offshore (oligotrophic) versus inshore (eutrophic), and in surface populations versus deeper populations during periods of thermal stratification. The sampling regime should be ship-based and provide appropriate spatial coverage: vertical transects of ≈ 10 depths, inshore-offshore transects, at least prestratification/poststratification seasonal comparison. Small volume samples (< 1 liter) are anticipated.

METHODS AND PLATFORMS

I have designed the methodological approach that I am taking specifically to avoid the artifacts that may arise from bottle incubations. We are using 2-D gel electrophoresis to examine proteins produced by cultured bacteria grown under specific physiological conditions. Our purpose is to identify proteins that can be used as general biomarkers of inorganic N/P utilization by bacteria. Appropriate proteins will be used for antibody production and these antibodies will be applied to natural assemblages of bacteria to assess the physiological status of the bacteria.

STRENGTHS AND LIMITATIONS OF PROPOSED RESEARCH

A strength of this methodological approach is the ability to assess the physiological condition of bacterial assemblages without prior incubation. Artifactual results due to bottle incubation continues to be a major problem in microbial ecology. Another advantage of this method is that it provides a means for subsequent identification of genes producing useful biomarkers (via protein sequencing and gene isolation). This has been a powerful approach for identifying genes associated with specific physiological abilities. This approach has not yet been heavily exploited in marine research. This latter work (gene identification) is beyond the immediate scope of this project but it will provide useful information to guide future research.

Potential limitations of my approach involve the significant lead time for the identification and isolation of proteins and the production and testing of antibodies. Another problem that could arise is the lack of conservation among diverse bacterial taxa of the proteins associated with inorganic nitrogen and phosphorus utilization. However, this conceptual approach has proven fruitful for other projects using similar methodology.

STATUS OF RESEARCH

I have recently hired a postdoctoral investigator on this project. It took more time than anticipated to identify a suitable candidate for this work, but the person I have hired is extremely appropriate and well trained for this work. At this time we have obtained suitable bacterial species for the culture work. We have tested these species in a variety of media designed to specifically elicit (or repress) proteins associated with inorganic nitrogen and phosphorus uptake and utilization. We are in the process now of performing and analyzing 2-D protein gels from bacteria grown in batch cultures. We will expand this work in batch culture to include continuous culture of these microorganisms within the next several weeks. Continuous culture will permit us to grow bacteria under specific conditions of limitation in order to examine changes in protein signatures with different physiological states. We have also recently acquired and installed a Sparc 10 SUN workstation equipped with software for the analysis and comparison of 2-D gels. Gel comparison is a key issue in distinguishing proteins that might be

appropriate for antibody production. This equipment (supported by Woods Hole Oceanographic Institution) will aid gel analysis tremendously.

Keywords: microbial, nutrients, immunofluorescence

**PRINCIPAL
INVESTIGATOR(S)**

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PROJECT TITLE

EXTENSION OF ^{210}Pb GEOCHRONOLOGY WITH INVERSE
NUMERICAL TECHNIQUES

AMOUNT OF FUNDING FY 1994: \$46 K

SUMMARY OF GOALS

The principal goal of this research is to significantly improve on the determination of sediment accumulation rates over conventional techniques. During the first two years of the OMP, the basic modeling procedure was successfully developed and tested. The goal now is to apply the procedure to determine age distributions with sediment depth for radioisotope profiles collected during the field portion of the OMP.

A) Assessing the Effect of Sediment Mixing on Age Determinations - Mixing caused by biological or physical processes, distorts or erases the signature of radioisotope decay in sediment profiles. Numerous studies have been conducted to understand mixing processes and from these, researchers have developed a variety of mathematical formulations to simulate mixing effects on radioisotope profiles. These models will be applied to a variety of synthetic radioisotope data profiles having known age distributions. The profiles will then be analyzed by the numerical procedure to determine the resultant effects on the age determinations caused by the different mixing models. Profiles will be created to represent a range of variable tracer strengths and variable sediment accumulation rates. These results will allow us to better define the practical limitations of determining sediment ages from disturbed radioisotope distributions.

B) Determining the Accuracy of Sediment Ages - Applying the procedure to a given radioisotope profile, we are able to determine a distribution of sediment ages at each sediment depth. The incorporation of probability/risk data interpretation techniques will allow us to rigorously define the accuracy limitations of the model determined sediment ages. This information will then be used to evaluate when the expenditure of resources for additional data collection will significantly improve the accuracy of a given age distribution.

C) Data Sectioning - The determination of accumulation rate distributions with depth in a sediment column exhibiting highly variable radioisotope concentrations is tedious and time-consuming. We must section the data into several pieces and analyze the pieces separately.

We will enhance the modeling code so that it will perform the sectioning procedure automatically and thus greatly reduce the amount of time needed to analyze complex data profiles.

SPATIAL AND TEMPORAL SAMPLING SCALES

This computer modeling project requires no independent field collection activities. The data to be used in this project will be provided through collaborations with other researchers in the OMP, principally, Dr.'s L.K. Benninger and P. H. Santschi. However, as previously mentioned, the determination of sediment ages from sediment core radionuclide profiles collected during the initial field studies will be used to direct where additional resources should be distributed. Sediment core sampling locations and sampling intervals will be defined to achieve the desired level of detail in the analysis of carbon burial histories.

METHODS AND PLATFORMS

Natural excess ^{210}Pb and weapons-fallout nuclides ($^{239,240}\text{Pu}$, ^{137}Cs) provide information on rates of sediment accumulation and mixing for the past 100 years. ^{14}C is used to determine accumulation rates over longer time-scales. The numerical algorithm will be used to derive accumulation rates from different tracer profiles to determine time variation in accumulation rates in sediment cores from shelf-rise environments off Cape Hatteras.

STRENGTHS AND LIMITATIONS OF PROPOSED RESEARCH

Strengths - This new procedure is a vast improvement over conventional approaches for determining sediment ages because it increases the number of data types from which realistic sediment ages can be derived. Also, the resolution of the depth distribution of sediment ages is significantly increased and the accuracy of these ages are well characterized. These improvements will allow the OMP to use information collected early on in the field investigations to determine where additional data is needed to achieve the desired spatial and temporal resolution. In addition, data collected during previous investigations on sediment accumulation rates in the MAB can be re-interpreted using the new procedure so that none of the historical investigations need be repeated.

Limitations - Tests conducted on synthetic profiles suggest that the procedure works best when sediment accumulation rates are variable (Carroll et al., in prep.). If the evidence suggests that sediment accumulation rates are constant at a location, we will apply both the new and conventional procedures.

In all cases, it is important to identify historical markers in the sediments, such as $^{239,240}\text{Pu}$ and ^{137}Cs , to improve the accuracy of the sediment age determinations. When a marker is not

available, the sediment age probability distribution functions are broad and thus the ages are poorly constrained.

It is important to recognize that the resolution of sediment age determinations will depend on the sampling interval chosen for the sediment cores. In environments where the magnitude and frequency of sediment accumulation rate changes is high, the number of sediment core sampling intervals must be increased accordingly. The frequency of changing sediment accumulation rates that can be resolved will be determined by the number of data points for each radionuclide profile and is defined by the Nyquist sampling frequency.

STATUS OF RESEARCH

For the previous two years, we have performed numerous tests to determine the strengths and limitations of this modeling procedure and have significantly improved the numerical algorithms used in the procedure. The numerical code has been completely re-written from the programming language "Fortran" into "C". During this transition, several errors in the code were identified and corrected. In addition many of the fundamental numerical analysis routines were improved. Paramount among these improvements was the development of a new convergence routine that considers the uncertainty associated with each data point in finding the optimum set of ages at each sediment depth (Carroll et al., submitted (a)).

Numerous synthetic data profiles with known sediment accumulation rates have been analyzed using the procedure (Carroll et al., submitted (a)) These tests have provided essential information on the kinds of radioisotope profiles that the procedure will assign with correct sediment ages. Historical data on radionuclide profiles from sediment cores collected from a variety of environments have also been analyzed (Carroll et. al., submitted (b)). While we continue improving the modeling procedure we are beginning to place more emphasis on locating and re-analyzing data that is currently available from previous investigations in the MAB region.

Keywords: sediment accumulation, sediment mixing, sediment ages, isotopes

**PRINCIPAL
INVESTIGATOR(S)**

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PROJECT TITLE

DETERMINATION OF OCEAN/ATMOSPHERE CARBON
DIOXIDE FLUX WITHIN OMP SURVEY AREA (PROPOSED
REVISED TITLE OF ORIGINAL PROJECT "DEVELOPMENT
OF GAS CHROMATOGRAPHIC SYSTEM FOR DISSOLVED
ORGANIC CARBON ANALYSIS IN SEAWATER")

AMOUNT OF FUNDING FY 1993: \$81 K

SUMMARY OF GOALS

The original goal of our project, to develop a GC-based technique for the analysis of DOC in seawater in order to provide insight into the apparent extra DOC which was then being observed by a number of workers using IR-based systems, was made essentially moot between the time our original proposal was written and the time it was funded. The controversy over the extra DOC was removed when it was recognized that the large blank associated with the catalyst used in the oxidation of the DOC was not being fully characterized; in addition, a GC-based system virtually identical to that which we had proposed was independently developed by a group at WHOI.

At the same time, it became obvious that one component of the carbon budget in the waters of the OMP field area (ocean/atmosphere CO₂ flux) was not being measured by any of the other investigators involved in the OMP. This is a measurement with which the Lamont group has considerable experience. With the encouragement of the OMP project managers at DOE, we have redirected our involvement in the program in order to fill the perceived gap.

Revised goal: To determine the magnitude of the carbon dioxide flux across the air-sea interface over the OMP survey area.

SPATIAL AND TEMPORAL SAMPLING SCALES

Determination of carbon dioxide partial pressure in surface seawater and overlying atmosphere needs to be carried out at least during four (preferably five or six) periods within a

year (not necessarily the same year) in order to resolve the anticipated large seasonality in the oceanic CO₂ sink/source condition. Early spring (pre-bloom) and late spring (post-bloom) measurements will allow the magnitude of the effect of the spring bloom to be determined, while summer, fall and early winter measurements will allow the rate of recovery from the bloom-induced drawdown of CO₂ to be determined. We do not at present have sufficient seasonal data to evaluate the temporal variability of the surface seawater pCO₂, but anticipate that it will be greatest at the time of the spring phytoplankton bloom and will vary more slowly during the remainder of the year.

Based on our preliminary measurements in the Cape Hatteras region and the shelf/slope area between Hatteras and Georges Bank, the surface seawater pCO₂ spatial variability is greatest in the near-shore environment, especially in the vicinity of estuaries, while the offshore areas show relatively low-frequency variability except in the waters affected by the Gulf Stream.

METHODS AND PLATFORMS

Quasi-continuous measurements of surface seawater CO₂ partial pressures will be made from one or more of the ships carrying out the hydrographic measurements in the OMP field area, as well as possibly from other ships operating within the area, with the exact number and timing of cruises selected to maximize the temporal and spatial coverage. Our IR-based system has been successfully operated during one cruise by essentially untrained technicians from another institution (BNL) without undue burden, demonstrating that personnel limitations need not limit the cruises in which we may participate. We presently operate several of these systems on assorted ships-of-opportunity, and anticipate having at least one system available to devote totally to this project.

In the event that the development of buoy-mounted pCO₂ sensors is successful, it will be vital to have comparison measurements made several times during the year, so that the close temporal spacing of the measurements made at these few fixed locations can be tied to the broad spatial coverage of the shipboard system. This may require the operation of a second IR system on the buoy-tending vessel.

STRENGTHS AND LIMITATIONS OF PROPOSED RESEARCH

The greatest strength of our approach is that it is based on a proven analytical technique (we have had a comparable system operating continuously on Lamont's vessel, the *R/V Ewing*, for more than two years); the new generation of solid-state IR analyzers has been shown to be highly reliable in the difficult shipboard working conditions.

The principal limitation of the approach is the restriction of data acquisition to periods when a suitably instrumented ship is operating within the survey area. Proxy measurements made

by remote sensing (sea surface temperature and color) cannot give sufficient accuracy to allow the CO₂ flux to be computed with the required accuracy.

STATUS OF RESEARCH

We have taken part in two scoping cruises within the OMP survey area, during April/May 1993 and 1994. During the first cruise, aboard the *R/V Gyre*, approximately 500 nautical miles of ship track within the survey area and a long transit south of the area (from Miami to Norfolk) were investigated. During this cruise, the profound impact of the discharge from Chesapeake Bay was noted, with one of the strongest CO₂ sinks ever measured in the marine environment being found on the northernmost of three sections run perpendicular to the coast just south of the entrance to the Chesapeake (pCO₂ approximately 115 μ atm, undersaturated by 70% with respect to the 350 μ atm of the atmosphere). Still lower values, down to approximately 55 μ atm, were found inside the mouth of the bay! The second cruise, aboard the *R/V Columbus Iselin*, was completed less than one month ago and consequently the data have not been thoroughly examined. Eight transects roughly perpendicular to the coast were made between Cape Cod and Cape Hatteras, in addition to longshore tracks nearshore between New Jersey and the Maryland/Virginia border and offshore between North Carolina and Georges Bank. With the exception of small areas in the nearshore regions north and south of Delaware Bay, one area off North Carolina, and Nantucket Sound, the entire area appears to be weakly to moderately undersaturated with respect to the atmosphere during this (presumably post-bloom) season. There was a strongly undersaturated region just south of the entrance to the Chesapeake, as in 1993, and an addition region of quite low surface pCO₂ was noted near the outfall from the Delaware Bay. In general, the degree of undersaturation over the shelf decreases from north to south, with waters undersaturated by more than 50 μ atm being common north of approximately 38 °N, while undersaturations of less than 50 μ atm predominate south of that latitude.

Keywords: carbon dioxide flux, pCO₂, infrared analyzer

**PRINCIPAL
INVESTIGATOR(S)**

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PROJECT TITLE

MEASUREMENT OF *SYNECHOCOCCUS* IN SITU GROWTH
RATES USING FLOW CYTOMETRY AND rRNA-
TARGETED PROBES

AMOUNT OF FUNDING FY 1994: \$128 K

SUMMARY OF GOALS

The ultimate goal of this project is to gain an understanding of the factors regulating the dynamics of picoplankton populations and the contribution of these populations to primary productivity in coastal environments. To this end, we are developing methods for the measurement of *Synechococcus* growth rates in the field, free from the potential artifacts associated with bottle incubations. Application of such measurements to coastal ecosystems would greatly improve our understanding of the rate of primary production by this important group of organisms, the role of different environmental factors in regulating this rate, and the importance of grazing and other loss terms in the overall dynamics of these populations.

Our immediate goals are:

- 1- To establish protocols for the preservation and flow cytometric analysis of DNA, rRNA, and protein in individual *Synechococcus* cells.
- 2- To establish the relationship between these biochemical parameters and growth rate under a range of environmental conditions, and among a variety of *Synechococcus* strains.
- 3- To test these protocols and relationships in the field by comparing the results with more traditional measures of growth rate and primary productivity.
- 4- To use the method to measure in situ *Synechococcus* growth rates in coastal ecosystems.

SPATIAL AND TEMPORAL SAMPLING SCALES

For the purposes of testing the approach, spatial and temporal scales that are likely to encompass the greatest change in the activity of *Synechococcus* populations would be most valuable. Thus, a seasonal series of short cruises (e.g. spring, summer, fall) would probably be preferred. Within each cruise, cross-shelf depth profile transects would be appropriate. In addition, 24- or 48-hour "diel" stations during which the water column could be sampled intensively (e.g. every 2-3 hours) would be invaluable for investigating the changes in population characteristics that are driven by the light:dark cycle.

METHODS AND PLATFORMS

Method development is a major part of this project, as reviewed briefly above. Our anticipated strategy for cruises would involve preservation of samples at sea, and detailed analysis of these samples back on shore. Therefore, we do not expect to require any specialized platforms. Nevertheless, for the purpose of developing sampling strategies during the cruise, and for routine monitoring of incubation experiments, we may request space for a ship-board flow cytometer.

STRENGTHS AND LIMITATIONS OF PROPOSED RESEARCH

The strengths of the approach we propose derive both from the independence of the method from the need for any incubation, and from the analytical power of flow cytometry. Although incubations are a necessary part of many biological oceanographic measurements, and the data derived from these measurements forms the basis for much of our knowledge about the regulation of microbial and phytoplankton communities, the potential artifacts that such incubations introduce are well known and widely recognized. By using the "biochemical state" of the organisms recovered from the natural environment as an estimator for growth rate, our approach holds out the promise of measuring in situ growth rates that are completely without incubation artifacts. The application of flow cytometry to this problem not only provides for rapid, quantitative, and objective analysis, but also allows such analysis to be made on a group-specific basis. Flow cytometry furthermore can be used to physically sort cells out of the sample, allowing further microscopic, biochemical or molecular analysis of the organisms of interest.

The major weakness of the approach is its dependence upon a uniformity in the relationship between the biochemical index of choice and growth rate under different environmental conditions, and among all the organisms of interest. Our laboratory experiments are designed to ascertain the degree to which this assumption is met among the *Synechococcus*. If the growth rate response is modulated by specific environmental parameters (e.g. temperature),

these can in theory be measured in the field and accounted for. If the response varies greatly among *Synechococcus* strains, we may be able to use flow cytometric signatures and molecular probes to narrow the taxonomic specificity of the assay accordingly.

STATUS OF RESEARCH

The improvement of preservation and analysis protocols is ongoing. We have maintained and sampled a series of light-limited cultures of *Synechococcus* strain WH-8101 growing at a range of rates, and expect to begin analyzing these shortly. In addition, we have begun to analyze the effects of a diel light:dark cycle on the parameters of interest in two *Synechococcus* strains. In the upcoming months, we will undertake a broad series of culture experiments comprising a number of *Synechococcus* strains growing under a range of temperature and light conditions in order to extend our understanding of the effects of these parameters on biochemical composition. We expect to continue these laboratory investigations (including nutrient-limited chemostat studies) through the 1994-1995 project year, and anticipate being ready for field trials at the start of the third project year (~ Sept '96).

Keywords: cyanobacteria, flow cytometry, rRNA, growth, phytoplankton

PRINCIPAL**INVESTIGATOR(S)**

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PROJECT TITLE

DEVELOPMENT OF AN AUTONOMOUS CO₂ SENSOR FOR
MOORING-BASED TIME-SERIES MEASUREMENTS OF
SEAWATER pCO₂

AMOUNT OF FUNDING FY 1994: \$86 K

SUMMARY OF GOALS

The primary goal of our DOE-OMP research has been to develop a fiber optic sensor for mooring-based measurements of seawater pCO₂. Specific goals during the initial 2 year funding period were the following : fully evaluate the sensor response characteristics using a wide range of operating conditions (temperature, reagents, flow rates, etc.), develop reliable and reproducible sensor fabrication techniques, design a sensor instrument based on the preliminary studies which minimizes size, complexity and power, test the long-term stability of the sensor both in the laboratory and in the field, and determine the sensor susceptibility to fouling and corrosion.

Our ultimate objective is to use the sensors to record CO₂ mixed-layer dynamics with high temporal and spatial resolution. This information will quantify the magnitudes of biological and physical forcings on sea surface CO₂ which will lead to a better understanding of the controls on CO₂ fluxes between the air and sea.

SPATIAL AND TEMPORAL SAMPLING SCALES

The sensor instrument has been designed to operate on a mooring for up to 3 months at two measurements per hour. We tentatively plan to deploy 2-3 instruments on the heavily instrumented biogeochemical mooring off Cape Hatteras. More instruments may be deployed along the cross-shelf transects depending upon funding and time. The half-hour sampling frequency will enable us to capture short term episodic events over the 3 month deployment.

METHODS AND PLATFORMS

The sensor operates by equilibrating a colorimetric pH indicator with the seawater CO₂ and recording the color of this solution in a fiber optic flow cell. The sensor is designed for autonomous operation on moorings although it may also be used on CTD's or towed platforms.

STRENGTHS AND LIMITATIONS OF PROPOSED RESEARCH

The sensor instrument is simple, durable, low power, and relatively inexpensive (\$5-6K). It operates with precision similar to the conventional Li-Cor infrared CO₂ analyzers and does not require gas-phase equilibration. The long-term stability is very promising although it has not yet been evaluated for a full 3 month period. Fouling was not a problem during low productivity periods (winter and spring 1994). We'll see what happens as the water warms up.

STATUS OF RESEARCH

An autonomous CO₂ sensor instrument has been designed, built, and extensively evaluated. Initial tests were performed in a shallow flow through seawater tank for a 12 day period. These results were very comparable to the Li-Cor/equilibrators data taken over the same period. The instrument has now operated continuously for three weeks (over 1000 measurements) suspended off the WHOI dock in two meters of water. These results also appear very good although they have not yet been fully examined. This instrument and perhaps one other will be prepared for a month-long deployment off Cape Hatteras in August 1994.

Keywords: carbon dioxide, pCO₂, moored sensors

**PRINCIPAL
INVESTIGATOR(S)**

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PROJECT TITLE

ASSESSING THE FACTORS CONTROLLING THE
MAGNITUDE, VARIABILITY, AND FATE OF PRIMARY
PRODUCTION ON OCEAN MARGINS

AMOUNT OF FUNDING FY 1994: \$524 K

SUMMARY OF GOALS

Although continental ocean margins represent only 12% of the surface area of the world's oceans, they account for between 25 and 45% of the ocean primary production. This production is a consequence of photosynthetic energy conversion of inorganic carbon to particulate organic carbon. The processes regulating the sources and fates of primary production are multiple and interactive; they include intrinsic biological processes and extrinsic physical, chemical, and biological processes. Anthropogenic activities can affect these processes both directly and indirectly. Overall this effort contains two projects, one of which focusses on the intrinsic biological regulation of phytoplankton photosynthesis and the other which focusses on the extrinsic factors related the magnitude and fate of production. The specific goals of these efforts within the Ocean Margins Program are:

- 1) To provide the basic (i.e., core) measurements of (a) primary production based on radiocarbon assimilation, (b) spectral irradiance, (c) chlorophyll a, (d) particulate carbon and nitrogen, (e) optical properties of the particulates, and (f) descriptions of species compositions for selected areas and times.
- 2) To develop primary production models based on parameters derived from (1) and in collaboration with Kolber and Wirick (using measurements of variable fluorescence) that can "fill in" for spatial and temporal gaps in ship-based sampling.
- 3) To assess the residence time, turnover time and natural death rate of phytoplankton on the continental shelf.
- 4) To develop and integrate new biological and molecular methods to diagnose factors intrinsically limiting phytoplankton photosynthesis and to use these techniques in conjunction with conventional methods to assess the importance of "bottom-up" and "top-down" controls.

SPATIAL AND TEMPORAL SAMPLING SCALES

The core measurements are ship-based and will be both survey and synoptic. The measurements of primary production are typically conducted daily using simulated in situ incubation methods.

Production models are based on large scale parameterizations of the entire continental margin and will be developed in conjunction with satellite measurements of ocean color. An attempt will be made to develop algorithms for both phytoplankton chlorophyll and primary production for the study area.

In conjunction with the moored and towed fluorometer measurements, we will follow the fate of phytoplankton in the near-bottom nepheloid layer. This effort will include both biophysical measurements of photosynthetic energy conversion efficiency as well as molecular biological measurements of specific proteins.

METHODS AND PLATFORMS

Most of the research is ship-based with a strong laboratory component to provide the basis for novel measurements. The core measurements are based on well established protocols, such as those used in the JGOFS program. We will not use HPLC for pigment analyses as we see no significant gain relative to the effort expended.

The biophysical methods focus on (1) variable fluorescence parameters, (2) low temperature (77K) fluorescence excitation and emission spectra, (3) thermoluminescence, and (4) fluorescence lifetimes.

The molecular approaches focus primarily on western blots and poly-acrylamide gel electrophoresis.

STRENGTHS AND LIMITATIONS OF PROPOSED RESEARCH

This research component provides the framework for developing all the carbon flux input for the OMP. It is an integrated ecological, biophysical and molecular ecological program.

The molecular biological and biophysical methods are still in a state of iterative testing and improvement. In particular, the molecular biological methods are not "stand alone".

STATUS OF RESEARCH

The conventional methods are well in hand and have been used for the past decade. Some of the biophysical methods need to be modified to improve signal to noise (i.e., in the case of thermoluminescence we require 1 μ g of chlorophyll for a measurement).

The basic understanding of the field measurements requires experimental laboratory research, which is ongoing.

Keywords: phytoplankton, primary production, chlorophyll

**PRINCIPAL
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PROJECT TITLE COASTAL TRANSPORT AND DIFFUSION

AMOUNT OF FUNDING FY 1994: \$208 K

SUMMARY OF GOALS

1. To understand the genesis of the low frequency baroclinic pressure field and its role in the generation of the low frequency baroclinic currents. The goal is to use monthly averaged temperature, salinity, and current data from moorings together with hydrographic survey results to look at the balance of terms in the very low-frequency momentum and mass conservation equations, and thus better understand the factors controlling the seasonal mean currents.
2. To further our understanding of the bio-physical interactions on the shelf. SEEP-II demonstrated that we could measure phytoplankton and zooplankton biomasses with some degree of accuracy over long periods. The use of a three-dimensional array of sensors should make it possible to unravel this convolution of processes over intermediate to large scales, and to study more directly the impact that mixing and shear have on the biological processes. This kind of study should also make it possible to study the spatial structure of the phytoplankton and zooplankton fields.
3. To study the kinematics and dynamics of the frontal eddies and filaments with the intent to quantify the cross-frontal transport that results from these possibly dominant processes. These features collectively may be responsible for the majority of the cross-frontal transport of mass, and suspended and dissolved constituents. These difficulties, however, can be quite simply addressed by satellite directed sampling with synoptic hydrographic surveys and TOYO/Seasoar high speed sampling.

SPATIAL AND TEMPORAL SAMPLING SCALES

1. The mean cross-isobath current shears observed in SEEP-II suggest isopycnal slopes of between 6×10^{-4} to 10^{-5} . The high isopycnal slopes apply to winter and sub-thermocline summer conditions. The lower slope values apply to the summer thermocline. With a sufficient vertical array of SEACATS to measure temperature and conductivity, the higher slope, (10 cm/km),

should be easily observable over about 5 kilometers, while the smaller isopycnal slopes of the summer thermocline, (1 cm/km), will require ten or more kilometer spacing. Vertical spacing of the SEACATS should be no more than 5 meters within the thermocline, while sub-thermocline and winter spacing could probably be about 10 meters, based upon the low vertical modal structure observed during SEEP-II and the thickness of the summer thermocline. ADCPs will be needed to provide adequate precision and vertical resolution for measuring currents.

2. Biological patchiness is not well defined on the shelf. There are conflicting ideas about whether there are dominant scales of patchiness, that is, peaks in the wavenumber spectra, or whether the spectra is "red". Horizontal spectra of fluorescence from an along-isobath (40 m) TOYO section from the MAY 1993 OMP cruise exhibit generally a "red" character, although there is a significant peak at about 10 km. We do not have any evidence yet on the cross-isobath fluorescence spectra. These results suggest that mooring spacing for biological studies should be no further apart than 5 km, and it would be better if the spacing were half that distance.

3. In the southern MAB frontal waves and meanders have been observed in satellite figures with alongshore scales 20 to 50 km, while filaments have been observed with alongshore scales of 10 to well over 100 km and offshore scales well in excess of 100 km. Vertical scales of these features are generally unknown. Temporal scales vary from a few days to a few weeks.

METHODS AND PLATFORMS

1. For examining the low frequency internal pressure field, we will need a three-dimensional array of moorings with sufficient horizontal spacing and vertical resolution to observe the mean tilts of the isopycnals, and with the ability to separate the high from the low frequency fluctuations. A minimum of four to five moorings will be needed although nine moorings would be desirable. The moorings should be in the vicinity of the 35 to 40 m isobath near 36° 30'N. Each mooring should be equipped with an ADCP and four to five SEACATs. Hydrographic surveys and satellite SST and color photos will be needed to bring the array into the larger shelf context and to identify anomalous conditions.

2. The bio-physical interaction study will also require ADCPs and SEACATs in a three-dimensional array of about five moorings but the spatial scale should be smaller, perhaps one half the size. The moorings will also require fluorometers with about the same vertical spacing as the SEACATs. Calibration net tows will be needed for converting acoustic backscatter intensity to biomass estimates, and to monitor the zooplankton species composition and feeding and growth rates. Productivity measurements from either or both shipboard incubations and fast repetition rate fluorometers will also be needed. The more frequent the shipboard sampling the better.

3. The study of frontal eddies and filaments will require satellite-guided shipboard surveys. The best alternative is a SEASOAR since it combines speed with a wide suite of instruments. A TOYO can be used instead, but the slower speed of the ship, 3 kts versus 8 kts, limits the areal

coverage and the synopticity. Standard hydrographic casts will also be needed because it will be important to measure the chemical constituents to distinguish water masses, and to estimate the amount of these constituents being exchanged across the front. Approximately four to five days of shiptime will be needed for each survey, with a total of perhaps four surveys during the program.

STRENGTHS AND LIMITATIONS OF PROPOSED RESEARCH

The studies outlined above are fairly robust in the sense that they utilize off-the-shelf or existing measurement equipment and techniques. The second goal, to measure bio-physical interactions, is the most difficult because of the difficulty of dealing with spatial inhomogeneities. However, it can be addressed using much of the array required in the study of the low frequency baroclinic field, and the two measurements would complement each other. The real limitation in the program is the imbalance between the equipment required and the equipment in the hands of the OMP investigators at the moment. At this point there are three ADCPs that are suitable, one at BNL and 2 (perhaps 3) at NCSU, four SEACATs (at BNL), and 15 fluorometers that are in need of refurbishment (at BNL) and two FRRs. A significant increase in hardware and manpower will be needed to make any of these ideas feasible.

For the shipboard surveys we have a CTD/Rosette and TOYO system that work very well. However, we have limited manpower to cover the number of cruises that are contemplated (four to eight) and to process the data. The TOYO has proven extremely useful, but it is limited in depth range (about 50 m) and speed (< 3 kts), and since it uses the same CTD that goes on the rosette, it puts at greater risk a vital piece of equipment needed by everybody on the ships. A SEASOAR potentially would address these issues, although it will require substantial effort to make it ready for shallow shelf work.

STATUS OF RESEARCH

To date in the OMP program we have participated in two cruises to the Cape Hatteras area, May 1993 and March-April 1994. The data from the May 1993 cruise, which included standard hydro- and chemistry casts and TOYO surveys, have been processed and archived. Some of this data was presented at the September OMP meeting. Work is under way on two papers, one on the general hydrographic and chemical distributions seen on the cruise, and another on the "cold band" eddies that were seen. The data from the March/April 1994 OMP cruise are being processed. On this latest cruise we deployed our bottom-moored ADCP and four SEACATs at a central mooring as part of a triangular array to examine the variability of the area and test the feasibility of making long- term, moored, dissolved oxygen measurements. The moorings will be retrieved near the end of June and the data processed over the next six months.

Keywords: hydrography, moored sensors, ADCP, TOYO, circulation

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PROJECT TITLE

ORIGINS AND FATES OF DISSOLVED ORGANIC MATTER
ALONG THE NEW ENGLAND CONTINENTAL MARGIN

AMOUNT OF FUNDING FY 1994: \$289 K

SUMMARY OF GOALS

1. We are characterizing the cycling of dissolved organic matter along the eastern U.S. coast, using samples collected on three offshore cruises. We are using a combination of concentration and isotopic measurements (DOC, DON, DO^{13}C , DO^{14}C , DO^{15}N , POC, PN, PO^{13}C , P^{15}N), as well as ancillary measurements of inorganic nutrients to develop our understanding of the offshore DOM cycle.
2. We are also performing mesocosm experiments (4 to 1000 l) that test controls of DOM formation and degradation. Of particular interest is the preferential storage and release of C and N species during DOM cycling. These experiments require time course measures of TCO_2 in addition to those mentioned above.
3. There is also considerable emphasis (50% of effort) in developing new techniques for measuring isotopic contents of DOM, i.e., DO^{13}C , DO^{14}C and DO^{15}N . We are trying to finish this work in our final year of current funding, which ends June 1, 1995.

SPATIAL AND TEMPORAL SAMPLING SCALES

We have participated in an April 1993 cruise to Georges Bank and an April 1994 cruise from Georges Bank to Cape Hatteras. We are hoping for a short 3-day cruise to Georges Bank this summer (July 1994).

In the next grant cycle we plan to work at both the macroscopic and mesoscopic scales: macroscopic scale being the entire Middle Atlantic Bight and mesoscopic being the process/box scale off Hatteras. Macroscopic sampling should include at least 2 cruises, and preferably three, over the spring-summer-fall period. Mesoscopic sampling should be conducted at least once during each season of the year. Experimentation initiated during mesoscopic cruises would entail daily resampling for an initial 2 week period followed with a monthly frequency thereafter.

We think working across larger shelf gradients (100+km) is essential for following DOM cycling and for comparing one shelf system to another (e.g., international comparisons).

METHODS AND PLATFORMS

Our methods center around water collected at sea and processed in our home laboratory. Hydrocasts and large volume pumping from ships are adequate for our purposes of obtaining water. Methods used are state-of-the-art. DON and DO^{15}N are being measured using UV oxidation as no HTOC method presently gives quantitative recoveries of added compounds in a variety of coastal waters.

STRENGTHS AND LIMITATIONS OF PROPOSED RESEARCH

The strengths of the combined isotope/concentration approach to understanding DOM cycling is the redundancy of multiple measurements on bulk DOM. A strength of our particular program is our interest in DON, which seems to be more dynamic than DOC. A limitation of the macroscopic survey approach is that sampling needs to be frequent, 2-4x/year, to characterize DOM dynamics across and along the shelf to the Hatteras region. We feel that repeated field sampling will probably better reveal natural patterns. So far, we have not had frequent ship time.

We are conducting mesocosm experiments to test controls of DOM cycling, as this is the kind of information necessary to construct simulation models of shelf C dynamics. Extrapolation of results to other shelf systems will be difficult if not impossible without the aid of mathematical models.

STATUS OF RESEARCH

DOM analysis

We have developed a reference, high temperature sealed tube method for DOC analysis, and have joined three intercalibration exercises designed to show how to and how not to analyze DOC (dissolved organic carbon). The overall conclusion of this community-wide analytical effort seems to be that the older, low temperature wet chemistry techniques for analyzing DOC (including persulfate and UV digestions), were largely adequate. The new high temperature techniques are, however, more rapid and, in the hands of experienced investigators, more precise.

We are continuing development of a DO^{15}N technique. As HTOC techniques do not give uniform recoveries, we are relying on UV oxidation both for concentration determinations and for the initial step in collecting N for isotopic determination.

Offshore cruises

We joined Dan Repeta and Tim Eglinton of WHOI in April 1993 for a short 5-day cruise to Georges Bank. DOC analyses did not show any marked changes across the Bank, in spite of large changes in algal biomass. We also collected water from Georges Bank and from over benthic cores to check how fast DOM might decay. Overall, rates of decay were very slow, declining less than 25% over six months of incubation.

In April of this year, we participated in a cruise from Georges Bank to Hatteras. Preliminary results show an increase in DOC near shore and to the south in the Middle Atlantic Bight, suggesting that DOM is exported from estuaries and then off the shelf in the Hatteras region.

In the upcoming summer cruise to Georges Bank, we will be searching for transient DOC increases that we expect at the end of phytoplankton blooms. Dynamics of DOM decomposition will be examined in water collected from various stages of bloom development.

Isotope methods development work

We have expended much of our effort developing routine, reliable methods for analyzing carbon and nitrogen isotopes in DOM. This effort is ongoing.

We are looking towards a renewal submission (Dec. 1 1993) that emphasizes DON dynamics both in the Hatteras region and along the entire Middle Atlantic Bight. We are interested in labile vs. refractory DON, DON export from estuaries, and rates of DON degradation in situ. The details of the DON cycle are poorly known compared to the growing knowledge about DOC, but DON appears more dynamic than DOC, and may partially control DOC distributions. Our last year of funding includes a heavy emphasis on developing methods for DON analysis.

Our renewal will also focus on elucidation of the controls of DOM production and decomposition and on the mechanisms that may lead to preferential recycling of N relative to C. Preferential recycling is critical to being able to sequester C on a long term basis.

At the scale of the entire Middle Atlantic Bight, our renewal will use a combination of concentration and isotopic measurements (DOC , DON , DO^{13}C , DO^{14}C , DO^{15}N , POC , PN , PO^{13}C ,

$P^{15}N$), as well as ancillary measurements of inorganic nutrients to develop our understanding of the offshore DOM cycle.

Samples will be obtained from the water column, sediments and water overlying sediment cores.

Keywords: dissolved organic matter, POC, DOC, isotopes

**PRINCIPAL
INVESTIGATOR(S)**

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PROJECT TITLE

CONTROL OF LIGHT SATURATED PHOTOSYNTHESIS BY
THE CONCENTRATION AND ACTIVITY OF RIBULOSE
BISPHOSPHATE CARBOXYLASE

AMOUNT OF FUNDING FY 1994: \$85 K

SUMMARY OF GOALS

The primary objective is to determine whether the maximum, light-saturated rate of photosynthesis (Pm) of phytoplankton can be predicted from measurements of the concentration and catalytic activity of ribulose biphosphate carboxylase. Specifically, we seek to determine the extent of variability in the maximum catalytic rate, the activation state and the ratio of Rubisco:chlorophyll a in marine phytoplankton. Variations in activation state of rubisco may result from physiological adaptation, light-activation, diel rhythms and interspecific (genetic) variability.

SPATIAL AND TEMPORAL SAMPLING SCALES

The appropriate time scales for sampling are those on which Pm varies. Processes which modulate Pm operate on time scales of hours (diel variability in Pm) and days (physiological adaptation). Thus, we wish to investigate within day and between day variability.

We require, at a minimum, morning, midday and evening samples from the top and bottom of the mixed layer and from the chlorophyll maximum layer (if there is one). We would like to sit on station to minimize spatial variability in process studies examining diel variability, but can accommodate spatial variability in surveys (if necessary, we can conduct studies of diel variability using samples incubated in controlled temperature deck top incubators).

It is imperative that this research be conducted in conjunction with fast repetition rate fluorescence investigations. FRR and Rubisco measurements provide complementary information. One goal of this research is to assess the relationship between regulation of the turnover time of whole chain electron flow and the regulation of rubisco activity.

METHODS AND PLATFORMS

Rubisco abundance will be determined by titration against the synthetic, noncompetitive inhibitor carboxyarabinitol bisphosphate (CABP) or by labelling with radioactive CABP or by immunological staining. The maximum activity and activation state will be measured *in vitro* using ^{14}C bicarbonate as a tracer. *In vivo* activity will be obtained from photosynthesis-light response curves determined from ^{14}C uptake using a photosynthetron. Photosynthetic pigments will be determined by HPLC.

All research will be ship-based, using conventional CTD sampling. Water volumes of 8-10 L will be required for PI curves, Rubisco and HPLC pigment determinations. Continuous access to surface water perhaps through a non-toxic on-line system would allow intensive temporal sampling appropriate to examining short term (including diel) variability.

Requirements include bench space and a dedicated fume hood in which radioisotope work can be performed.

STRENGTHS AND LIMITATIONS OF PROPOSED RESEARCH

Rubisco is one of the most abundant enzymes in phytoplankton. It is the enzyme which catalyzes CO_2 assimilation and as such is a key point in the regulation of the rate of photosynthesis. The principle strength is the contribution of this research to a mechanistic understanding of variations in Pm and turnover time for photosynthetic electron. These variations occur in response to time of day and physiological history with respect to light and nutrients.

Limitations: The technique is time consuming and thus the number of samples that can be processed is limited. There are likely to be interspecific variations in extraction efficiency, activation state and maximum catalytic activity (in fact, one goal to the research is to assess the extent of these variations.)

STATUS OF RESEARCH

This research is at the proof of concept stage. To date, laboratory results with the diatom *Thalassiosira weissflogii* and *Phaeodactylum tricornutum* have demonstrated: (1) The activation state of Rubisco is variable and this variation is correlated with the diel variability of Pm. (2) The activation state changes in response to light-dark transitions, deactivating in the dark to 15% of the maximum level observed in the light. (3) The rates of activation and deactivation of Rubisco in diatoms have very similar time constants to those observed in higher plants. (4) The kinetics of activation and deactivation of Rubisco have the same time constants as *in vivo* ^{14}C uptake. We are currently attempting to optimize extraction procedures and modify existing protocols to obtain quantitative measurements of Rubisco concentration in diatoms.

Keywords: phytoplankton, photosynthesis, ribulose biphosphate carboxylase

**PRINCIPAL
INVESTIGATOR(S)**

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PROJECT TITLE

THE DYNAMICS OF CARBON EXCHANGE IN
VERTICALLY STRATIFIED COASTAL
BACTERIOPLANKTON COMMUNITIES

AMOUNT OF FUNDING FY 1994: \$125 K

SUMMARY OF GOALS

Evidence suggests that bacterioplankton communities may be stratified, and that major taxa in displaced communities can undergo downward shifts in growth rate. The overall goal of this project is to determine whether vertical stratification in bacterioplankton communities affects carbon transport.

The short-range experimental goals of this project are: (1) to identify bacterial taxa which are abundant in the OMP study area; (2) to determine whether these taxa are vertically stratified; (3) to determine whether physical events which mix stratified communities cause specific taxa to decline in growth rate or enter stationary phase.

SPATIAL AND TEMPORAL SAMPLING SCALES

Current sampling methods limit bulk hybridization techniques to about 4 stations per transect across the continental shelf in the OMP study region. Samples for vertical profiles are collected at 10 meter intervals. About 10 liters of water are required per sample. Two hours are needed for onboard processing of a vertical profile consisting of 4 - 7 samples. Vertical sampling is not possible at every site in transects at this time. Hence, stations in the center of the study region and on the edge of the continental shelf have been chosen for repetitive sampling.

METHODS AND PLATFORMS

Bacterioplankton samples are collected with Niskin bottles and standard filtration methods. Total plankton nucleic acids are prepared at shore labs. Hybridization to radioactive oligonucleotides complementary to ribosomal RNAs is used to measure the abundance of specific

bacterioplankton taxa. Other methods, based on microscopic visualization of single cells, are under development in collaboration with Drs. Paul Kemp and Paul Blum.

STRENGTHS AND LIMITATIONS OF PROPOSED RESEARCH

The strength of this approach is the ability to measure significant elements of community structure which were previously unknown. The main limitation is that current methods are time-consuming. However, the pace of new developments is quick, with both the speed and accuracy of methods increasing.

STATUS OF RESEARCH

Members of the bacterial group, the "SAR11 cluster", have been identified in samples collected at the OMP study site. A suite of probes specific for the SAR11 cluster have been designed and tested. A 16S rDNA clone library (> 100 clones) prepared from Cape Hatteras bacterioplankton assemblages is being screened to identify additional bacterioplankton taxa. The most abundant of these taxa will become the focus of probe development and future study. In May, 1994, over 100 samples were collected at the study site (in triplicate). These samples will be used to verify the numerical importance of dominant bacterioplankton, and to test the reproducibility of methods.

Keywords: microbial, taxonomy, rRNA, rDNA, vertical stratification

**PRINCIPAL
INVESTIGATOR(S)**

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PROJECT TITLE

THE XANTHOPHYLL CYCLE ENZYMES IN
CHROMOPHYTE ALGAE AND PHYTOPLANKTON:
STRUCTURE AND SIGNIFICANCE

AMOUNT OF FUNDING FY 1994: \$0 K

SUMMARY OF GOALS

This project examines the detailed mechanisms that influence photosynthetic performance in marine phytoplankton, specifically, nonphotochemical quenching of chlorophyll fluorescence. This process is thought to dissipate excess excitation energy and limit the risk of photoinhibition or photodestruction of pigment-protein complexes. One of the faster quenching components correlates kinetically with establishment of a pH across the thylakoid membrane and the dark-reversible de-epoxidation of a xanthophyll. The product (zeaxanthin or in chromophytes, diatoxanthin) is the presumed quencher or quenching amplifier. Two enzymes function in this cycle but information on their location, mode of action and structure is scant for higher plants, and essentially lacking for phytoplankton. The goals of this project are: 1). to discover novel inhibitors of the enzymes so that the status of the cycle can be "locked in" upon harvest, for later shipboard analysis. These inhibitors will be used in collaborative work with Dr. Paul Falkowski to evaluate photoprotection by the xanthophyll cycle in situ, 2). the isolation, cloning and sequencing of the above enzymes as a basis for genetic manipulation e.g., selective gene transfer, gene deletion or limitation of expression through synthesis of antisense message.

SPATIAL AND TEMPORAL SAMPLING SCALES

We propose a temporal sampling schedule that includes several 24-hr time series stations occupied during different seasons. The effect of xanthophyll cycling and nonphotochemical fluorescence quenching on phytoplankton photosynthesis is poorly understood. However, we suggest these processes may be most important in surface waters during bright, sunny days when zeaxanthin or diatoxanthin production is likely maximized. The spatial sampling scale will be coordinated with other OMP investigators.

METHODS AND PLATFORMS

The in situ photoprotective effect of xanthophyll interconversions will be estimated from sampling vertically through the euphotic zone, by triggering collection bottles containing cycle inhibitors. Deckboard analysis of net photosynthesis (as O_2 evolution) and gross photosynthesis (from pulse fluorometry) will be performed at irradiances at, above and below those determined at the depth of sampling. Comparison with the performance of uninhibited phytoplankton samples should provide a meaningful assessment of any protective role for de-epoxidized xanthophyll cycle components (diatoxanthin and zeaxanthin). It will also assess any disadvantage accruing from the presence of these quenchers at low irradiance.

STRENGTHS AND LIMITATIONS OF PROPOSED RESEARCH

The use of selective inhibitors of the deepoxidase such as dithiothreitol, or of ionophores that collapse the transmembrane pH gradient, have provided considerable insight into the relationships between xanthophyll cycling, energy-dependent quenching, and photosynthesis in higher plants, and to a limited extent in phytoplankton. However, a convenient chemical inhibitor for the epoxidase is not presently available. Zeaxanthin (or diatoxanthin) is converted to violaxanthin (diadinoxanthin) in the light as well as in the dark by an enzyme that uses NADPH and molecular oxygen, and thus, can be blocked by anaerobiosis. The use of chemical inhibitors would be more advantageous than anaerobicity in blocking epoxidation, since the local environment of the thylakoid membrane is inevitably exposed to photosynthetically-derived O_2 . However, to be effective in providing meaningful information on the role of xanthophyll cycling in ocean margins, the selected inhibitors must be equally potent in a wide variety of species across varying taxonomic lines.

STATUS OF RESEARCH

Two classes of antimycotic agents, known to block the epoxidation of squalene in fungi and mammalian tissues, have been tested for their ability to inhibit zeaxanthin epoxidase in barley chloroplasts. Epoxidation was monitored by illuminating samples to accumulate zeaxanthin then inhibiting deepoxidation by simultaneous darkening and addition of the ionophore nigericin. Analysis of the magnitude and kinetics of the slow absorbance decrease at 505 nm, indicative of violaxanthin formation from zeaxanthin, demonstrated that the allylamines terbinafine and naftifine, and the thiocarbamate tolnaftate are potent inhibitors of zeaxanthin epoxidation. The concentrations yielding 50% inhibition of initial epoxidation rates were 4.6 μM tolnaftate, 6.0 μM naftifine, and 10.6 μM terbinafine. The 505 nm absorbance increase during actinic illumination, which reflects net accumulation of zeaxanthin, was apparently enhanced in the presence of these inhibitors, and an initial lag phase was abolished. Ascorbate-loading of chloroplasts in the presence of 20 μM tolnaftate yielded the highest observed rate of absorbance increase at 505 nm during actinic illumination. The dynamic interaction between epoxidation and deepoxidation in intact chloroplasts is remarkable in that epoxidation seems to compete effectively when

zeaxanthin is minimally available, at the onset of illumination. The sensitivity of zeaxanthin epoxidation to inhibitors of squalene epoxidation suggests some degree of sequence homology between the corresponding enzymes. Analysis of the effects of these antimycotic agents on diatoxanthin epoxidation in chromophyte algae is underway.

Keywords: phytoplankton, chlorophyll, fluorescence, xanthophylls, enzyme sequencing

**PRINCIPAL
INVESTIGATOR(S)**

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PROJECT TITLE

BURIAL, REMINERALIZATION AND UTILIZATION OF
ORGANIC MATTER AT THE SEAFLOOR UNDER A
STRONG WESTERN BOUNDARY CURRENT

AMOUNT OF FUNDING FY 1994: \$123 K

SUMMARY OF GOALS

The overall objective of this research is to quantify the role of seafloor processes in the biogeochemical cycling of elements in the shelf system. Specific goals within this context include:

1. Quantify the export of carbon from the southern MAB by determining the deposition rate of organic materials on the adjacent continental slope and rise.
2. Determine the exchange rate of nutrients and carbon between the sediments and water column of the southern MAB.
3. Identify the processes that control sea floor exchange rates and determine their spatial and temporal variability.

SPATIAL AND TEMPORAL SAMPLING SCALES

Benthic processes and exchange are variable on all length scales. Activities of individual macrobenthic organisms may alter processes over distances of less than 1 cm while the physical dynamics of the overlying bottom water may alter net depositional patterns on scales of 1-100 kilometers. Temporal variability is primarily driven by seasonal changes but may also be influenced on shorter time scales by physical processes such as wind events. Sampling is (or will) be conducted on all of these scales to assess the role of benthic processes in carbon cycling in the OMP study region.

METHODS AND PLATFORMS

Studies will be based primarily on sediment samples obtained by cores and by in situ benthic flux chamber incubations. Sediment and pore water analyses will be performed on the core samples to assess reaction rates and processes.

STRENGTHS AND LIMITATIONS OF PROPOSED RESEARCH

Sea floor studies take advantage of the physical framework provided by the bottom sediments to identify organic matter transformation processes and quantify reaction and sea floor exchange rates. Because bottom sediments provide a fixed framework in which to interpret concentration measurements and benthic chamber incubations, reaction rates and exchange rates can be more accurately assessed than in settings where such a framework does not exist.

Pore water and benthic exchange studies are limited by sampling techniques. Presently, there are no pore water measurements that may be performed in a remote, continuous manner, analogous to the continuous, mooring-based measurements in the water column. Pore water measurements are presently limited to shipboard procedures and may be obtained at a rate of 3-5 profiles per day. In situ benthic flux chamber measurements are limited by incubation length, estimated to be .5 day at shelf locations and 2 days on the slope.

STATUS OF RESEARCH

Slope pore water measurements and in situ benthic flux incubations have been performed in an attempt to estimate and determine the location of off-shelf export. Plans for this summer expedition include continued slope and rise measurements and preliminary shelf sampling.

Keywords: carbon deposition, benthic fluxes, nutrient fluxes, benthic cores

**PRINCIPAL
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PROJECT TITLE

MEASUREMENT OF MARINE MICROBIAL GROWTH.
MICROBIAL PROCESSES IN COASTAL MARINE SYSTEMS

AMOUNT OF FUNDING FY 1994: \$432 K

SUMMARY OF GOALS

Carbon cycle: The best overall approach to carbon cycling is biogeochemical, e.g. oxygen and DIC measurements. Within that framework, predictive capacity for individual component processes has to be developed to model or extrapolate to changed conditions in the future. Predictive capacity for processes cannot be developed from climatologies, which are all that are available for bacterial process measurements. High-resolution measurements of bacterial processes must be obtained, at a level relevant to the metabolic capacity of bacteria to respond to environmental conditions. As a first cut, this means bacterial taxa or taxon- crossing functional groups (whichever reflects the distribution of metabolic capabilities better). Growth rate is a reasonable first approximation of the activity of specific taxa/functional groups, although later work will clearly need to shift to gene- expression approaches.

Goals: Develop improved capability to measure bacterial growth. Develop mechanistic and predictive understanding of environmental factors controlling bacterial growth at molecular/ metabolic level.

SPATIAL AND TEMPORAL SAMPLING SCALES

Frequency of sampling/sample throughput: Sampling pace is not limiting and samples are effectively immortal in storage. Sample processing is shore-based, currently at about 15 samples/week/person. The image analysis system is expected to double sample throughput (30/week/person).

METHODS AND PLATFORMS

Research strategy: Measurements of rRNA content targeted to dominant members of bacterial community. Implementation of cooled-CCD camera/image analysis system to increase sensitivity and sampling throughput.

Rationale: Empirical relationship between rRNA and growth rate; control of bacterial growth rate seems to be interpreted through regulation of rRNA synthesis and concentration.

STRENGTHS AND LIMITATIONS OF PROPOSED RESEARCH

Sensitivity and limitations: Currently rRNA is measured in 100-ribosome increments starting at 100-200 ribosomes per cell. This includes ca. 2/3 of all cells in field samples. Cooled-CCD camera with image analysis will be more sensitive. Currently, multiple probes are used, but it will probably not be practical to design more than 2-3 probes specific to a given taxon. The method will probably not be useful for measuring rRNA in the least active cells in the field.

Other information: Need hydrography, chl, O₂, DOC, nutrients to interpret bacterial growth as a consequence of these properties. A measure of labile DOC could be especially useful. Need direct respiration measurements to constrain growth rate estimates.

STATUS OF RESEARCH

Status: Concept is proven and procedures have been applied in field studies. Program is now focusing on learning to interpret the data. Next applied goal is implementation of taxon-specific measurements in mixed communities (primarily design and testing of probes). We measure rRNA in individual cells, however, the rRNA-frequency data are entirely new and we have no notion how to interpret them as yet.

Caveats: RNA:growth rate relationship is not constant. We are not sure yet what influences the relationship; current efforts are focused on RNA regulatory behavior, taxon-specific differences, biovolume effects, and temperature. Available field data point to taxon-specific applications rather than any universal application to whole bacterial communities.

Keywords: microbial, growth, rRNA

**PRINCIPAL
INVESTIGATOR(S)**

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PROJECT TITLE

DEGRADATION OF PARTICULATE ORGANIC MATTER IN
COASTAL OCEANS: THE CHITINASE SYSTEM AT A
MOLECULAR LEVEL

AMOUNT OF FUNDING FY 1994: \$99 K

SUMMARY OF GOALS

The overall goal is to estimate concentrations and degradation rates of chitin in the water column of coastal oceans and to examine the contribution of chitin to the carbon and nitrogen budget of heterotrophic bacteria. Chitin is produced by many marine organisms and is thought to be abundant, although there are few direct measurements. In addition to being important per se, chitin may prove to be a good model substrate for understanding degradation of particulate organic matter by bacteria.

We can attack this question nearly immediately with methods already developed. Our immediate goal is to test these methods in controlled experiments. These methods include published procedures for measuring chitin concentrations and turnover rates. We need to see if we can put these methods together and show that we can truly measure chitin degradation.

Another goal of the proposed work is to develop molecular approaches for examining chitin degradation. The first step in this work is to obtain more information about naturally-occurring chitinases (the enzyme mainly responsible for the hydrolysis of chitin). We intend to isolate these chitinase genes, sequence them, and use this sequence information to synthesize PCR primers. These primers will be used then to assay chitinase gene amounts in coastal waters. The sequence information alone will be valuable because few chitinases have been sequenced and none from "natural" bacteria. Our first goal is to see, within an order of magnitude, if chitinase gene amounts correlate with chitin concentrations and degradation rates.

SPATIAL AND TEMPORAL SAMPLING SCALES

We can collect samples about every 5 km or every 4 hours.

METHODS AND PLATFORMS

The methods involve taking water samples with CTD or GoFlo bottles and doing analyses in the lab, both shipboard and on land. We intend to do many experiments in local waters off Delaware from small boats and ships of opportunity. After we are confident about our methods, we will look for space on OMP cruises in the later stages of this project (late 1995 or 1996).

STRENGTHS AND LIMITATIONS OF PROPOSED RESEARCH

Given that we know so little, I suggest that any information collected about chitin in coastal waters (even Delaware's) will prove interesting and important. Similarly, we know so little about chitinases from natural marine bacteria that any molecular information will provide insight into the degradation of particulate organic matter. The overall strength then of the proposed work is that it will make progress on two fronts (hopefully simultaneously): 1) the ecological and oceanographic questions involving chitin fluxes; 2) the molecular basis of chitin degradation.

Our present ignorance is our main limitation. We know so little that we need to test methods and to obtain more sequence information before molecular methods can be applied to the oceanographic problem of estimating degradation rates of chitin.

STATUS OF RESEARCH

We have tested methods for measuring chitin concentrations and turnover rates and are now poised to begin estimating degradation rates. Within a couple weeks, we will begin a regular sampling program of measuring concentrations, degradation rates and bacterial production in order to address the first oceanographic goals of examining the importance of chitin in carbon and nitrogen budgets.

On the molecular side, we are in the middle of sequencing a chitinase from a marine bacterium. Our next goal is to isolate natural DNA and to see if we clone a natural chitinase.

Keywords: chitin, microbial, gene sequencing, PLR primers

**PRINCIPAL
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PROJECT TITLE

FAST REPETITION RATE (FRR) FLUOROMETER FOR
MAKING IN SITU MEASUREMENTS OF PRIMARY
PRODUCTIVITY

AMOUNT OF FUNDING FY 1994: \$312 K

SUMMARY OF GOALS

The goal of this project is to understand what controls photosynthesis on the continental margin near Cape Hatteras. The scientific objectives of this project are:

- 1) To quantify primary productivity in the Cape Hatteras region using FRR measurements of the photosynthetic parameters. These measurements will be made from moored buoys and on process cruises using moored, shipboard, profiling and towed FRR fluorometers of our design.
- 2) To develop models that predict the dynamics of primary productivity from the temporal and spatial distributions of the photosynthetic parameters.
- 3) To continue development of the FRR methodology and instrumentation to increase our ability to measure and understand the dynamics of primary production.

This OMP project will significantly improve the understanding of the carbon cycle in Cape Hatteras region by providing time series and high-resolution sections of the photosynthetic parameters and primary production. In addition, laboratory experiments investigating the relationship between fluorescence will lead to an improved understanding of the dynamics of photosynthesis in marine phytoplankton.

SPATIAL AND TEMPORAL SAMPLING SCALES

OMP Moored Array: We propose to deploy five FRR fluorometers in the moored array to measure photosynthesis. The separation between instruments will be 5-10 m in the vertical and

5-10 km in the horizontal. The instruments will be sampled at 10-minute intervals and will have an endurance of 3-4 m.

TOYO and Surface Mapping: We propose to make FRR measurements on all TOYO deployments. The resulting high- resolution profiles have a vertical resolution of 1 m, a horizontal resolution of ~1 km and a repeat cycle time of 5-10 minutes. In addition, a shipboard FRR fluorometer may be used to provide continuous near-surface maps of the photosynthetic parameters.

CTD Profiling: On process cruises we propose to make in situ FRR measurements on all CTD casts. These profiles will have a vertical resolution of 1-3 m, and their horizontal resolution will be determined by the station spacing, which is expected to be 1-15 km.

Discrete Sampling: On process cruises we propose to make FRR fluorescence measurements on discrete samples collected as part of our investigations of photosynthesis.

METHODS AND PLATFORMS

Platforms: This project includes both mooring and shipboard support. We propose to deploy five FRR fluorometers in the moored array and will require 1 berth on mooring cruises. On processes cruises this project will require 1-2 berths.

Methods: Primary production is a highly dynamic phenomenon because it is governed by nutrient availability, solar irradiance, and temperature. Physical processes distribute the phytoplankton cells, and the subsequent transformations occur over a wide range of time and space scales. Since the relative importance of various physical and biological processes varies seasonally and spatially, the OMP will require an array of moored instrumentation to measure photosynthetic parameters of phytoplankton over long temporal scales, as well as the ability to rapidly map large areas to acquire a mesoscale distribution of photosynthetic parameters at selected time intervals.

A fluorescence based methodology called fast repetition rate (FRR) fluorometry will be used to rapidly measure the rates of photosynthesis and quantify the fundamental parameters controlling these rates. The FRR methodology is based on sound, biophysical principles of light absorption, photochemistry, and carbon fixation. The basics of FRR methodology have been verified in both laboratory and field conditions, and the methodology continues to evolve. Instrumentation implementing the FRR methodology has been used in the TOYO, profiling, and discrete modes for more than a year.

STRENGTHS AND LIMITATIONS OF PROPOSED RESEARCH

The FRR fluorometer strengthens the OMP program because it can be used to measure the distribution of the photosynthetic parameters with high spatial and temporal resolution.

Information gathered from these measurements will help provide the capability to estimate the dynamics of primary production and identify its limiting factors, and simultaneously constrain the phytoplankton pool and the carbon fluxes surrounding it.

The success of the moored application will depend on our ability to control the fouling of the optical windows in the moored instrument. We have developed brush mechanisms to keep optical windows clean, and we plan to install such a mechanism on the moored version of the FRR fluorometer.

STATUS OF RESEARCH

Several FRR fluorometers were field-tested during the 1993 OMP cruise. The discrete, underway mapping, profiling, and TOYO configurations performed as expected. FRR estimates of primary production correlated well with ^{14}C measurements. A moored FRR fluorometer was deployed near Cape Hatteras in March 1994. Following its recovery in July 1994, the instrument's performance will be critically evaluated with regard to long-term calibration.

Two laboratory investigations are being conducted as part of the FRR project. There is a continuing effort to relate fluorescence and photosynthesis by comparing FRR fluorescence to oxygen production. A fluorescence time-resolved study on the biophysics of photosynthesis using the BNL National Synchrotron Light Source is continuing as well. The results of this effort will be used in further development of the FRR protocols.

In Phase II of OMP, we propose to become a basic research component, directed toward understanding the mechanisms of photosynthesis, the relationship between photosynthesis and fluorescence, and the effects of environmental factors on the functioning of photosystem II in marine phytoplankton.

Keywords: photosynthesis, primary production, fast repetition rate fluorometer

**PRINCIPAL
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PROJECT TITLE

PARTICULATE INORGANIC CARBON

AMOUNT OF FUNDING FY 1994: \$0 K (BNL Laboratory Directed Research and Development (LDRD))

SUMMARY OF GOALS

The goal of this project is to examine the distribution of particulate inorganic carbon (PIC) and its role in the carbon cycle on the continental margin near Cape Hatteras. Effects of PIC on alkalinity and $p\text{CO}_2$ will be studied. Laboratory experiments will be conducted in parallel to identify the environmental factors favorable to calcification in the major calcifying phytoplankter, *Emiliania huxleyi*. The scientific objectives are as follows: 1) Quantify the amount of particulate inorganic carbon (PIC) in the water column over a seasonal cycle. 2) Assess the abundance of calcified and naked coccolithophorids, in particular *Emiliania huxleyi*, the dominant calcifying phytoplankton species in the study region. 3) Conduct laboratory experiments to identify the environmental factors that promote calcification in coccolithophorids. 4) Survey images of water-leaving radiance from the coastal zone color scanner (CZCS) to identify potential blooms of coccolithophorids.

SPATIAL AND TEMPORAL SAMPLING SCALES

Most of the research can be conducted from ships. Samples can be collected on most of the OMP hydrographic and biological cruises, and either stored for lab analysis or processed at sea. Proper coverage requires cross-shelf transects and seasonal distribution of the cruises, including fall and winter sampling.

METHODS AND PLATFORMS

PIC will be measured from filtered seawater samples using a Gas chromatography technique (GC-FID) which has a detection limit for CO_2 of the order of 10-10 moles. The filtered samples will be rinsed, dried, acidified and the CO_2 captured for analysis. Using 0.2 pg of carbon per coccolith and a minimum coccolith concentration of 10^4 l^{-1} (non-bloom conditions), there should be a background PIC of 10^{-9} moles/l so that even low PIC could be detected by

filtering 1 l of water. During a moderate-size coccolithophorid bloom, the concentration of PIC would increase by three or more orders of magnitude.

The coccolithophorid *E. huxleii* has been reported as one of the dominant phytoplankton within the study site. However, only calcified cells can be positively identified as *E. huxleii* using conventional microscopy techniques (light and electron microscopy) so that this species, which has both motile and non-motile naked life stages, may be far more abundant than previously assessed. The abundance of the coccolithophorids will be assessed by conventional microscopy, which uses polarized light to detect calcified cells. Both naked and calcified cells will be detected by immunostaining with *E. huxleii* cell surface antibodies, and using HPLC to monitor the concentration of the pigment 19' hexanoxylfucoxanthin that is characteristic of coccolithophorids.

STRENGTHS AND LIMITATIONS OF PROPOSED RESEARCH

The PIC pool, comprised mainly of CaCO_3 , is an important component of the total carbon pool. The process of biocalcification, which leads to PIC accumulation, is an important biogeochemical pathway within the carbon cycling. In contrast with other phytoplankton growth that results in the production of POC and drawdown in pCO_2 , coccolithophore growth can cause a significant drawdown of alkalinity and relative increase in pCO_2 . The influence of a coccolithophorid bloom on air-sea exchange of CO_2 has been recently documented in the Sargasso Sea near Bermuda (U.S. JGOFS Bermuda Atlantic Time-series Study, 31° 50' N, 64° 10' W) where a short-term increase in surface PCO_2 of 30 f atm reduced the net annual air-to-sea flux of CO_2 by approximately 25%. Direct measurements of PIC during the OMP field year will help obtain a complete carbon characterization of the carbon cycle at the study site. While major blooms of coccolithophores have not been reported for the study area, sampling has been limited and *E. huxleii* is a dominant species in the phytoplankton assemblages (most abundant identified species in offshore shelf region) throughout the year. Blooming conditions could become favorable, as they did in February 1992 at the BATS site. The process of calcification is poorly understood biochemically and it is therefore essential to complement the field studies with laboratory experiments in order to identify the environmental conditions favorable to calcification.

Since OMP sampling will be limited to seasonal cruises, a transient increase in the abundance of coccolithophores could easily be missed. However, their effect on the chemistry of seawater could persist for several months and modify the annual net transfer of atmospheric CO_2 into the surface waters.

STATUS OF RESEARCH

All of the proposed measurements can be accomplished using equipment available in OASD. The proposed field work will complement laboratory studies already underway on the

process of calcification in *E. huxleii*. Calcifying and non-calcifying strains of *E. huxleii* are currently being maintained in culture at OASD.

Keywords: inorganic carbon, phytoplankton, coccolithophorids

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PROJECT TITLE

TIME-SERIES OBSERVATIONS OF FINE SCALE
DISTRIBUTIONS OF PIGMENT FLUORESCENCE IN THE
BENTHIC BOUNDARY LAYER USING A MULTI-SENSOR
FIBER OPTIC FLUOROMETER

AMOUNT OF FUNDING FY 1994: \$135 K

SUMMARY OF GOALS

In support of the Department of Energy (DOE) Ocean Margins Program's (OMP) goal of quantifying cross-shelf and shelf-slope exchanges of particulate organic matter, we have developed a multi-sensor fiber optic fluorometer capable of resolving fine-scale gradients in pigment fluorescence in the benthic boundary layer. Our goals include relating temporal variations in pigment fluorescence to physical (near-bottom velocity and shear) and biological (water column productivity and pigment distributions, activities of benthic fauna) processes being studied by other OMP investigators. Questions to be addressed by this research include the following: 1) Is there significant resuspension and redistribution of pigments in the benthic boundary layer? 2) Can gradients in pigment fluorescence be related to velocity and density profiles in the benthic boundary layer? 3) How are gradients in pigment fluorescence related to particle properties and distributions in the benthic boundary layer? 4) Is there a relationship between variations in upper water column productivity and pigment concentrations and variations

in the benthic boundary layer? 5) What is the quantitative significance of cross-shelf and along-shelf transport of pigments?

SPATIAL AND TEMPORAL SAMPLING SCALES

The use of fiber optic sensors provides for great flexibility in the spatial scales that can be sampled with a single instrument, ranging from centimeters to 10's of meters. The instrument is currently configured for sampling eight depths at intervals within a range of 5 m above the bottom. Horizontal sampling scales will be limited by the number of instruments and the number of platforms which can accommodate them (see below).

The instrument is capable of high sampling rates (up to 1 Hz), although lower sampling rates will extend battery life during deployments. With current battery design and power requirements, we estimate a nominal 1200 h (50 d) of operation with sampling rates of 10 min per h.

METHODS AND PLATFORMS

The autonomous instrument is capable of measuring and storing fluorescence data at eight different depths. The present system is contained within an 18 cm diameter, 1 m long aluminum casing with 16 fiber optic feedthroughs at one end. Autonomous operation and data logging are achieved using a Tattletale 7 (TT7) with a 120 Mbyte hard drive and interface board on which additional logic has been constructed. The TT7 is interfaced with the Ocean Optics PC1000-ADC board (500 kHz/12 bit). Excitation light is provided by 4 strobe units (EG&G). The light is filtered using a Schott BG-28 blue transmission filters. Strobe intensity is monitored with separate fibers going to a phototransistor. Detection of fluorescence is achieved with two Hamamatsu photomultiplier tubes equipped with 676 nm narrow bandpass filters (Edmund A43, 140, 1 in diam.).

The multi-sensor fluorometer will be deployed on the eastern U.S. continental shelf north of Cape Hatteras near the Duck site for a 4-6 week period in July-August 1994 in conjunction with the CoOP Pilot study. Our instrument will be mounted on the BASS bottom tripod system of Williams and Churchill. The BASS tripod system provides an ideal platform for deployment of the fiber optic fluorometer array. Variations in fluorescence can be interpreted in the context of data collected with other BASS sensors including thermistors, CTD, acoustic travel time current meters, optical backscatterance (OBS) sensors and laser diffraction particle size measurements. This will permit an assessment of the extent that resuspension and lateral flows result in transport of pigments associated with the benthic boundary layer. Ideally, calibration of OBS and fluorescence measurements to particulate organic carbon can be made using simultaneous measures of organic matter from time-series samplers.

STRENGTHS AND LIMITATIONS OF PROPOSED RESEARCH

The strengths of the proposed methodology include the capability for time-series observations of fine-scale distributions of pigment fluorescence using relatively inexpensive hardware. Time-series observations are essential for characterizing episodic events that may contribute significantly to lateral fluxes on the shelf. The low power demand of our instrument should allow for extended autonomous operation. Another strength is the ability of our instrument for precise sampling of fluorescence in the benthic boundary layer. Estimates of flux of particulate pigments in the boundary layer require knowledge of the vertical integral of the vector velocity at each height times the pigment concentration. Since both the velocity and concentration vary with height, it is necessary to measure both at enough heights to resolve the variability. The use of fiber optics allows for precise placement of fluorescence sensors at multiple depths that will minimally interfere with other sensors.

One of the potential weaknesses of this and any optical sensor is the potential for bio-fouling. Bio-fouling will be considered as a potential factor influencing the fluorometer signal response. This will be especially critical during long deployments. Previous strategies for dealing with fouling problems have included both mechanical (Whitledge and Wirick, 1986) and chemical (Whitledge and Wirick, 1983; Dickey et al., 1991) methods. In addition to use of an anti-fouling clear organo-metallic polymer (tributyl tin), designs are being studied for construction of a hydraulic system for periodic scrubbing of the optical sensors as well as "self calibration" of the instrument.

Calibration of the autonomous instrument represents a major challenge to the research effort. A considerable proportion of our efforts will focus on instrument calibration and factors which may modify this. We are currently evaluating instrument calibration and drift in controlled laboratory conditions. Factors to be considered include effects of ambient light and temperature. In addition, the possible influence of changes in particle concentrations and size distributions will be examined empirically and through the use of Monte Carlo simulations. In field situations, the instrument will be cross-calibrated on the basis of contemporaneous measurements of fluorescence from other fluorometers and of organic matter and extracted pigments from water samples. In addition, the development of a "self calibrating" capability during deployment using a stable fluorescence reference is being explored.

STATUS OF RESEARCH

A successful field test of the multi-sensor instrument in the benthic boundary layer was recently conducted aboard the R/V Pelican in May 1994. Observations were conducted in an area near the Mississippi River outflow plume where significant accumulation of pigments in sediments has been observed (Turner and Rabalais, 1994). Preliminary results revealed large variations in near bottom fluorescence in 10-15 m of water. The variations appeared to be too large to be explained on the basis of in situ growth, and will be evaluated in the context of other measurements including acoustic doppler velocimetry, transmissometry and water column pigment

distributions. Experiences on the cruise were constructive in identifying areas of focus for further optimization of hardware design and software algorithms.

Keywords: fluorescence, fiber optic fluorometer, benthic boundary layer, pigments

**PRINCIPAL
INVESTIGATOR(S)**

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PROJECT TITLE

ADSORPTION CONTROL OF ORGANIC CARBON BURIAL
IN OCEAN MARGIN SEDIMENTS

AMOUNT OF FUNDING FY 1994: \$104 K

SUMMARY OF GOALS

1. Interpret the regional (estuary, shelf, slope, rise) patterns of organic matter (labile and refractory) accumulation and burial in sediments in the context of grain size of the sediments. Do initial accumulation and eventual burial in the sediments follow similar grain size relationships, or does initial accumulation from the water column show less grain size correlation than eventual burial? Can grain size be used as an integrating variable for accurate large-scale mapping of organic carbon (OC) burial? Does grain size normalization allow resolution of important organic carbon sinks (e.g., slope off of Hatteras vs. slope to the north?)? These goals can be achieved through two types of study:

a. Small scale ridge and swale study. Assess the seasonally changing relationship between small-scale spatial heterogeneity of sediments (ridge and swale topography) and organic matter accumulation. This study would be done in conjunction with process-oriented benthic groups (e.g., Aller, Jahnke), who would study the metabolism of the organic matter. I would examine indicators of fresh, labile vs. bulk, refractory organic matter. Collaboration with groups such as the biomarker team (e.g., Eglinton et al.) would be useful.

b. Map regional grain size relationships of OC burial throughout the estuary-shelf-slope-rise region. This work can be done over many years, because the parameters are long-term integrating ones. As many cores as possible need to be radiometrically dated for sedimentation rate by other persons (e.g., L. Benninger) in the program, or myself, via subcontract. If OC burial is at concentrations similar to the monolayer-equivalent level, such as I have been finding in many shelf-slope regions in my current project, then I will be able to make statements to the effect that long-term organic carbon burial is controlled completely by the rates and distribution patterns of mineral sedimentation.

2. Terrestrial vs. marine organic matter in estuaries. One of the interesting questions arising from my current study is if terrestrial organic matter is delivered to the oceans at

monolayer-equivalent levels, and if so, then is it replaced on mineral surfaces by marine organic matter once entering the marine system? To address this question I would monitor riverborne sediments entering the Chesapeake Bay, and examine cores in the transition region of the Bay. I would hope to get some collaborative work done by the biomarker group. I would also like to do some laboratory experiments that test for the mechanisms of this replacement (e.g., metabolism of terrigenous organic matter vs. desorptive removal).

SPATIAL AND TEMPORAL SAMPLING SCALES

1a. Small scale ridge and swale study. I envision seasonal cruises to sample sites in a restricted set of shelf environments within close proximity (ca. 100 km), done during a year of intensive, process-oriented water column and benthic work. Similar process-oriented studies might be done in other areas in subsequent years (e.g., slope depocenter, canyons).

1b. Map regional grain size relationships of OC burial. I envision collecting cores from throughout the estuary-shelf-slope-rise region around the study area. Each site need be visited only once, and timing is not tied to any process study. On the order of 10^1 - 10^2 cores will be collected.

2. Terrestrial vs. marine organic matter in estuaries. The riverine sampling of suspended particulate matter would be done from shore over the course of a year, with emphasis on periods of major sediment delivery (spring flood). Coring would be done on one estuarine cruise.

METHODS AND PLATFORMS

1a. Small scale ridge and swale study. Coring to be done from shipboard, in conjunction with other benthic group scientists in this process study. I would measure various parameters of sedimentary organic matter, including measures of labile material (protein, perhaps plant pigments if no one else is doing it) and total organic matter (C, N), and sediment specific surface area as a textural indicator.

1b. Map regional grain size relationships of OC burial. Box or Kasten cores to be collected from shipboard (larger ships offshore, smaller vessels in coastal/estuarine regions). I will measure total organic matter and sediment specific surface area, and would need radiometric dating for sedimentation rates.

2. Terrestrial vs. marine organic matter in estuaries. Riverine samples will be collected from shore, so no platform is needed. Estuarine coring will be done from small vessel. I will measure total organic matter and surface area, and will subcontract for stable isotope measurements.

STRENGTHS AND LIMITATIONS OF PROPOSED RESEARCH

Organic carbon deposited to the sediments is metabolized over a long time, with decreasing rates over time. My approach will provide estimates and assist with explanations for organic carbon preservation in various sedimentary regimes on various time scales. My ability to explain the terrigenous-to-marine transition will be limited by the ability of stable isotopes (and biomarkers if they are included in the study) to quantitate the various mixes of terrestrial vs. marine organic matter. Similarly, the measures of labile organic matter, while the best available (I believe), offer only imperfect estimates of this fraction of the organic matter present.

STATUS OF RESEARCH

Progress over the last two years has seen the publication of Mayer (1994, *Geochim. Cosmochim. Acta* 58:1271-1284), which demonstrates clearly that the amount of organic carbon burial in many continental shelf environments around the world is correlated to the adsorption capability of the sediments. An empirical case for this control derives from the organic carbon-surface area correlations, with a slope of 0.9 mg-OC per m² of mineral surface area. A causal mechanism for protection of organic matter at this concentration is hypothesized to be protection from hydrolytic enzymes of heterotrophs by adsorption into pores too small to allow enzyme entry, a hypothesis which is supported by pore size data on the mineral surfaces. A second paper is now in press (*Chem. Geol.*), which extends the relationships of the first paper to a number of terrestrial soil and marine continental slope and deep sea environments. The second paper explores the implications of this adsorption control in more detail, extending its impact to temporal as well as spatial variations in organic matter burial.

We are continuing to collect and analyze samples in ocean margin environments to better delineate the spatial extent of these correlations. A few cores from the Hatteras slope area have been analyzed (cores courtesy of L. Benninger), and show consistency with the global data set (albeit with remarkably high organic carbon concentrations at the slope depocenter). A frequent question when I present this work at meetings regards the meaning of the term "monolayer-equivalent". I am therefore spending considerable effort this year exploring how to measure the extent of organic matter coverage on mineral surfaces. My approach involves using the different enthalpies of adsorption of argon and nitrogen gases on mineral vs. organic surfaces. There is some success, though the outcome is yet uncertain.

Keywords: carbon burial, carbon accumulation, isotopes, grain size, sediment topography

**PRINCIPAL
INVESTIGATOR(S)**

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PROJECT TITLE

PHOTOSYNTHETIC ENERGY CONVERSION EFFICIENCY
IN INDIVIDUAL PHYTOPLANKTON CELLS FROM "PUMP
AND PROBE" FLOW CYTOMETRIC MEASUREMENTS

AMOUNT OF FUNDING FY 1994: \$236 K

SUMMARY OF GOALS

To develop an instrument to determine photosynthetic energy transfer efficiency in different groups of phytoplankton from natural samples, and to use this instrument to characterize the nutritional status of these cells in different oceanographic regimes. Such an instrument will supplement measurements of active fluorescence by existing Fast Repetition Rate instruments, which measure photosynthetic parameters on bulk water samples.

SPATIAL AND TEMPORAL SAMPLING SCALES

We will measure samples from depth profiles at stations in nearshore to open ocean waters, and over seasonal time scales (on process study cruises). We are not planning for *in situ* sampling or moored operation.

METHODS AND PLATFORMS

The methods employed will be the pump-probe flow cytometer under development, which will require a small to mid-size oceanographic vessel (e.g., RV Oceanus). The samples will be analyzed at sea, but not *in situ*. Comparisons with FRR measurements and other productivity measurements will be an important part of the study.

STRENGTHS AND LIMITATIONS OF PROPOSED RESEARCH

The main strength of the approach is that it will allow us to examine individual phytoplankton cells (or particular groups within the phytoplankton) as opposed to bulk water samples containing variable mixtures of cells and detritus, etc. This should increase the

usefulness of the data, and allow us to better interpret the results of bulk water FRR measurements. The main limitations of the pump/probe flow cytometer are those of sensitivity, in that at present we are able to measure only the larger phytoplankton cells. In addition, we are not planning to try to make *in situ* measurements, so we will obtain potential rates rather than real-time information such as is obtained by FRR fluorometry. The measurements will be more limited in scope, but more highly resolved in terms of organisms, than the FRR measurements.

STATUS OF RESEARCH

We are still in the early stages of development of the instrument, and in fact we are now trying a new approach (photon counting) to increase sensitivity so we can measure small as well as large cells, so actual construction has not yet begun. We still plan, however, to have a working instrument in the next year (even if it only works for large cells), to participate in the field year.

Keywords: phytoplankton, flow cytometer, nutritional status, fluorescence

**PRINCIPAL
INVESTIGATOR(S)**

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PROJECT TITLE

IMPACT OF PROTO- AND METAZOOPLANKTON ON THE
FATE OF ORGANIC CARBON IN CONTINENTAL OCEAN
MARGINS

AMOUNT OF FUNDING FY 1994: \$274 K

SUMMARY OF GOALS

Metazooplankton remove particulate organic carbon from the ocean by ingestion, and they produce organic carbon by growth, reproduction, excrementation and excretion. Our approach is to quantify ingestion by abundant metazooplankton not only of phytoplankton carbon but also of heterotrophic nano- and microplankton which have been shown repeatedly to be a major food source of planktonic copepods. Simultaneously, biomass, egg production and fecal pellet production will be quantified for the same abundant to dominant zooplankton taxa.

The nano/microplankton component of this joint project includes both laboratory technical development and field applications. Technique development comprises two subsections: improvement of our existing color image analysis system to quantify composition, size distribution, and carbon biomass of photosynthetic and heterotrophic nano- and microplankton; and evaluation of the efficacy of an immunochemical assay to measure predation by (gelatinous) macrozooplankton on protozoan zooplankton. These tools would subsequently be used off Cape Hatteras to determine (1) the apportionment of living and non-living POC into phytoplankton and heterotrophic components, (2) carbon ingestion by nano- and microzooplankton, and (3) the transfer of the living POC to higher trophic levels.

SPATIAL AND TEMPORAL SAMPLING SCALES

Our Multiple Net System (MNS) was designed to quantitatively collect larger and smaller metazooplankton. It now can be operated to a depth of 300 m, can hold up to 6 nets, and can be deployed in 20 to 30 minute intervals, depending on the maximum depth of sampling; we can easily sample at 5 km intervals. Zooplankton grazing experiments aboard ship should be conducted over 24 h, with 2 experiments run simultaneously. The CritterCam, an *in situ* movie camera, arrived several weeks ago. It will be repeatedly field-tested during the second half of 1994, and is intended to be deployed eventually for observations over days to weeks to quantify

concentrations of abundant zooplankton.

Nano- and microplankton samples are derived from shipboard Niskin bottle samples. (1) Preparations for plankton composition and biomass require 5 minutes/sample aboard ship but 1-2 hours each to analyze back in the lab. Thus the scales of sampling are purely labor-dependent. Repetitive sampling of stations at 5 km intervals can be accomplished. (2) Experimental incubations to measure grazing rates require 24 hr. Experience in JGOFS indicates that two experiments per day (i.e., two different depths at one station or one depth at two stations, depending upon whether the ship is in transect- or time-series mode) is possible, depending upon labor constraints (bunk space). (3) Immunochemical assays of predation on microzooplankton require vertical profiles of predator and prey. The time frames for sample collection and analyses are as in (1), while the post-cruise laboratory procedures are somewhat more time consuming. A 5 km spaced cube could be sampled but less frequently than (1), e.g, once every 3-4 days.

METHODS AND PLATFORMS

Sampling requires capture of water parcels from shipboard platforms. Grazing of phytoplankton (primary production) and other small heterotrophic plankton will be measured aboard ship from incubation experiments, using changes in algal pigments (HPLC, fluorometry) and changes in carbon biomass, composition, and size distribution of the plankton communities. Analysis of plankton carbon will be conducted ashore using a state-of-the-art color imaging system and inverted microscopy. Ingestion of microzooplankton carbon by macrozooplankton using immunoassays will also be determined ashore from field-collected samples.

STRENGTHS AND LIMITATIONS OF PROPOSED RESEARCH

Metazooplankton:

Strengths. Sophisticated feeding rate quantifications are needed to determine actual feeding rates of abundant metazooplankton. Since it is obvious that nearly every stage of a copepod is potentially an omnivore (being able to feed on autotrophs and heterotrophs), respective experiments quantifying such performances need to be made, even if they would be time-consuming. Simultaneously, with sophisticated feeding studies, we will determine metazooplankton growth rates.

Limitations. The traditional microscopic analysis of net-collected metazooplankton is laborious and time-consuming. They need to be made until we possess the technology to quantify respective genera and stages directly *in situ*, or from flow-by samples on board ship. They are needed because biomass does not distinguish between calanoid and cyclopoid copepods, tunicates, carnivorous coelenterates and chaetognaths, each of these taxa contributing in a different manner to carbon flow in the ocean.

Nano microplankton:

Strengths. Grazing by nano- and microzooplankton is now recognized as usually the dominant biological loss term for primary production in most aquatic ecosystems. Combined measurements of grazing rate and composition/biomass has proven to be a powerful tool elsewhere (e.g. JGOFS) in assessing not only the production, cycling, and fate of plankton POC, but also as a tool to interpret data from more "black-box" approaches (^{14}C , thorium budgets, sediment traps). The use of color image analysis provides increased accuracy, precision, and sample processing speed (i.e., greater spatial and temporal resolution). The immunochemical approach will, if successful, provide the first estimates of transfer of microzooplankton carbon to larger predators.

Limitations. The major problem in evaluating the roles of nano- and microplankton is their small size. Technology is not yet available to quantify their composition, abundance, size distribution, (carbon) biomass, grazing, or growth rates *in situ*. Until that time, detailed spatial and temporal mapping of plankton will be manpower-dependent.

STATUS OF RESEARCH

Our studies on pelagic tunicates resulted in several original data: we were successful in obtaining two complete life cycles of the doliolid *Dolioletta gegenbauri* under experimental conditions, simulating *in situ*. One complete life cycle lasted 28 days at $\sim 45\mu\text{gC/l}$ of phytoplankton. Feeding rates at these food levels surpassed previously obtained values by factors 2 to 3 which is attributed to providing near optimal conditions for these doliolids. During the following months we intend to obtain rates on sexual and asexual reproduction as well as on growth at various natural food levels to calculate carbon consumption and production by a doliolid assemblage.

Improvements to our imaging system are either in place or will be completed by the end of the current funding period. We have upgraded our software, added an x-y-z-axis controller and a motorized microscopic stage. A state-of-the-art cooled integrating Optronics color CCD will replace the existing (excellent but aging) Hitachi CCD. Antibodies to several ciliate antigens (cell surface and whole cell) have been prepared and are presently being tested for specificity and cross-reaction with other plankton. We have developed an incubation method to measure ingestion of both autotrophic and heterotrophic plankton by metazooplankton.

We participated in the May 1993 and March 1994 "biology" cruises, and we will be aboard the June 1994 cruise to Cape Hatteras. Data analysis from the first cruise is complete. Results were presented at the September 1993 OMP PI meeting. A poster will be presented at the ICES Symposium on Zooplankton Production in Plymouth (August 1994) and a joint manuscript with BNL scientists (Verity et al.) has been recently written. Another manuscript comparing March, May and June-July data is anticipated in early 1995.

Keywords: microzooplankton, metazooplankton, grazing, phytoplankton, biomass, composition

**PRINCIPAL
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PROJECT TITLE

CELL-SURFACE PROTEINS AS MARKERS FOR THE
PHYSIOLOGICAL STATE OF COASTAL
PHYTOPLANKTON

AMOUNT OF FUNDING FY 1994: \$143 K

SUMMARY OF GOALS

The goals of the sampling program are to 1) Begin to validate the use of polyclonal antibodies to specific cell surface proteins as indicators of physiological state of the phytoplankton. 2) Use the antibodies in more of a survey mode once they have been characterized. It should be noted that essentially every identified cell surface protein/organism combination will go through both phases. We expect to have at least two to three antibodies ready to test in 1995.

SPATIAL AND TEMPORAL SAMPLING SCALES

The spatial and temporal scales will be relatively modest in phase 1 including only a few depth profiles. We hope to work out conditions for using preserved cells which may be organism specific. Phase 2 will involve a more systematic survey of the sampling area. Further information on species distributions within the sampling site and isolation of specific strains will help in the choice of sampling scales.

METHODS AND PLATFORMS

Possibly one liter samples from bottle casts will be required. The samples will be assayed using fluorescently labeled antibodies and fluorescence microscopy on board ship and/or on preserved samples in the laboratory.

STRENGTHS AND LIMITATIONS OF PROPOSED RESEARCH

The strengths of the approach are that we can begin to look at the physiological characteristics and state of specific phytoplankton cells within a population and the different factors limiting their growth. Ecosystems are complex and the factors limiting one group of organisms might not be limiting others so that the single species/single cell approach will be extremely valuable and will eventually provide a more mechanistic understanding of the whole ecosystem. On the negative side identifying candidate cell surface proteins as markers, purifying them, identifying their function, and making antibodies to them is extremely time consuming and these kinds of proteins may not always be present in all organisms.

STATUS OF RESEARCH

The first goal of the project was to work out the best conditions for labeling phytoplankton cell surfaces with biotinylating reagents in order to detect cell surface proteins. The method has been essentially perfected. We have since used the method to look for nitrogen-limitation specific cell surface proteins in *Emiliana huxleyi*, one of the most commonly found marine phytoplankton, and in two diatom (*Thalassiosira*) species. We have found such a protein in *Emiliana* but not in the diatoms. The diatoms, however, have proteins found in healthy, but not stressed, cells suggesting that antibodies to these proteins could also be used as indicators of cell state. The candidate N-limitation protein in *Emiliana* is found in the membrane/cell wall fraction of the cells and is about 80 kDa in size based on SDS-PAGE. It is found in three strains of *Emiliana* from widely different locations including a strain from the Gulf of Maine, the site of yearly blooms of this organism. We have also found a candidate for a phosphorus limitation induced protein in *Emiliana* so that antibodies to the two proteins could be used to look at P vs N limitation of this organism in the field. We are continuing to screen other coastal phytoplankton for marker proteins and we are beginning the purification of the 80 kDa protein for antibody production. The second goal of the project was to purify a cell surface L-amino acid oxidase from a coccolithophorid. This protein allows certain phytoplankton to grow on amino acids as a nitrogen source and it is induced in some organisms under N limitation. The protein seemed like a good candidate for a test of the approach of using cell surface antibodies to look at environmental stress. We purchased and set up a Gradifrac system from Pharmacia and have used it to purify the amino acid oxidase using gel filtration and ion exchange chromatography. Antibodies are currently being made. These antibodies will be fluorescently labeled and used to work out the best conditions for cell surface protein detection in seawater and will also be used on field samples in the future. Some additional work is needed to complete the characterization of this protein.

Keywords: phytoplankton, antibody, cell surface proteins, physiological state

**PRINCIPAL
INVESTIGATOR(S)**

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PROJECT TITLE

REGULATION OF PHOTOSYNTHETIC CARBON FIXATION
ON THE OCEAN MARGINS

AMOUNT OF FUNDING FY 1994: \$58 K

SUMMARY OF GOALS

The goals are to 1) understand regulation of ribulose biphosphate carboxylase (RubisCO) in phytoplankton cultures in response to light regime 2) determine regulation of RubisCO in response to light during nutrient limitation in these cultures 3) to determine mechanisms of RubisCO regulation in natural populations of phytoplankton on the ocean margins in the Gulf of Mexico and 4) to measure regulation of RubisCO in phytoplankton of the Hatteras System. Two goals are laboratory-based, and two are ship-based.

During the first year, the Tabita lab is primarily responsible for objectives 1 and 2 of the overall project (see above). Specifically, we have initiated experiments to probe the regulation of RubisCO in response to different light and nutrient limitation conditions in representative marine cyanobacteria and diatoms. These studies have been performed to understand the factors that control RubisCO gene transcription and RubisCO activity, and to relate these molecular perturbations to changes in the marine environment that influence overall CO₂ and carbon metabolism.

SPATIAL AND TEMPORAL SAMPLING SCALES

Participation in DOE cruises is highly desired. We would be interested in spatial sampling across the shelf in conjunction with the spring bloom at least 4 stations along a transect (for vertical profiles). During a second cruise, diel regulation and regulation under various light regimes is required for us to complete our DOE mission. These studies last 24-48 hr/diel with sampling every 4 hr.

METHODS AND PLATFORMS

Laboratory Studies. To pave the way for studies with organisms that are more difficult to culture and extract, we have begun our investigations with marine cyanobacterial strains with which we have some experience. *Anabaena* sp. strain CA is a marine nitrogen fixing organisms that we isolated, along with several other strains, 17 years ago. It is representative of a group of marine cyanobacteria found on the ocean margins and it grows well in the lab. We have worked out all the basic techniques for measuring RubisCO transcripts from this organism under both light and nutrient limitation. These studies resulted in the significant observation that the RubisCO genes (*rbcLrbcS*) and the gene (*rca*) that specifies an enzyme that modulates RubisCO activity *in vivo*, namely RubisCO activase, are independently regulated at the level of transcription. These studies are relevant to understanding the ability of RubisCO to fix CO₂ which drives the ecosystems of the ocean margins.

For the study of RubisCO regulation, four types of biological measurements are required: 1) measurement of transcriptional regulation, by extraction and quantitation of *rbcL* mRNA 2) measurement of RubisCO enzyme activity in extracts of cells 3) determination of the amount of RubisCO protein, determined immunologically and 4) determination of whole cell carbon fixation. These methods provide closure on all mechanisms of regulation for this enzyme. Additionally, we will amplify, clone, and sequence some *rbcL* genes from natural populations of phytoplankton and from specific cultures provided to us by collaborators and of our own isolation.

For the studies described herein, approximately 20x20' deck space is required. A vessel with a CTD with a fluorometer probe, light meter, and rosette sampler is required. In terms of shipboard lab facilities, 2 fume hoods are required, one for mRNA extraction, one for filtration of samples treated with DEPC (diethyl pyrocarbonate). Adequate space for radioisotope work is also required. Deck space for incubators is also needed. For these studies to be performed in conjunction with other components of the DOE OMP, a reasonably large vessel will be required.

STRENGTHS AND LIMITATIONS OF PROPOSED RESEARCH

Strengths. Clearly one of the greatest strength of this project is the capability of measuring phytoplankton gene expression by mRNA isolation and quantitation. A second strength is the amplification of *rbcL* genes for cloning and sequencing, which will enable determination of the types of organisms responsible for oceanic carbon fixation.

Limitations. A potential limitation of the project deals with uncertainty on the appropriateness of our cyanobacterial gene probe to detect oceanic picoplankton *rbcL* genes. Although we have had a high degree of success using the *A. nidulans* (= *Synechococcus* PCC6301) probe, some recent studies in the Tabita lab indicate that it only weakly hybridizes with *Synechococcus* WH7803 (although this organism may not be representative of oceanic picocyanobacteria). Our preliminary studies on the Pacific *Prochlorococcus* by sequence analysis suggests that it would

hybridize well with our *A. nidulans* probe. We are examining production of probes from oceanic picocyanobacteria we have in culture to mitigate this potential limitation, as well as using our diatom probe to get the "golden" clade of phytoplankton.

A second concern is how to do diel studies. Current studies with deck top incubators suggest that conditions in these deteriorated in as few as 12 hr, which make studies of any duration difficult. The alternative, tracking a water mass by a drogued buoy, does not enable manipulation of light regimes and does not preclude vertical migration of the phytoplankton population out of the zone of sampling.

STATUS OF RESEARCH

As mentioned above, we are making good progress in lab studies with phytoplankton isolates and in field studies of natural populations in terms of the regulation of RubisCO. In October, we participated in laboratory studies on the regulation of *rbcL* using Pacific *Prochlorococcus* cultures in the lab of Dr. Lisa Campbell, University of Hawaii. The regulation of *rbcL* was studied through light and dark cycles of a 52 hr diel study. The results of these experiments are forthcoming as the data is being processed. We participated in a cruise in September to study regulation of RubisCO in vertical profiles and during a diel experiment. We have planned a cruise for August, funded by a NSF project. This cruise will examine regulation of RubisCO in the field, as well as look for transcriptional regulation throughout the water column. A third cruise is scheduled off the Hatteras area in summer of 1995, again funded by NSF.

A major publication based on this work will be submitted to a leading journal by the time this report is received. Most important, however, is the fact that our experience with *Anabaena* has provided us with the necessary expertise to tackle aspects of regulation in open oceanic strains, organisms which are much more difficult to cultivate. To assist in these efforts, we have isolated an oceanic unicellular strain from the Gulf of Mexico, obtained on a cruise in September of 1993. This strain grows fairly rapidly (unusual for such organisms) both on liquid and solid marine growth media. The latter point is particularly important for any future studies on the genetic regulation of CO₂ fixation in marine cyanobacteria. Most interesting is the fact that this new isolate may be capable of fixing nitrogen. If confirmed, this will be an exciting observation, for it may mean that such organisms contribute to the nitrogen budget of the oceanic environment as well.

Keywords: photosynthesis, ribulose biphosphate carboxylase, nutrient limitation

**PRINCIPAL
INVESTIGATOR(S)**

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PROJECT TITLE

SEDIMENT MOTIONS AND BOTTOM BOUNDARY LAYER
DYNAMICS OVER THE MIDDLE ATLANTIC BIGHT SHELF
AND UPPER SLOPE

AMOUNT OF FUNDING FY 1994: \$221 K

SUMMARY OF GOALS

L.J. Pietrafesa is presently conducting pilot studies of sediment dynamics over the Middle Atlantic Bight (MAB) in the vicinity of the Cape Hatteras Confluence (CHC) including the mouths of estuaries, the shelf and the slope, as part of the Department of Energy Ocean Margins Program in concert with G. Weatherly (FSU) and J. Churchill and S. Williams (WHOI). Being studied are processes which effect sediment motion and in particular, the processes which determine rates of vertical transport of dissolved carbon dioxide and organic matter and particulates to and from the bottom by turbulent mixing resuspension and particulate sinking and vertical motions induced by BBL convergences; especially during periods of storm activity when both surface waves and currents are maxima.

SPATIAL AND TEMPORAL SAMPLING SCALES

SPATIAL

a. Vertical

1. Benthic Boundary Layer (Several tens of centimeters to meters)
2. Surface Boundary Layer (Meters to tens of meters)
3. Estuarine Circulation and Plumes (Meters to tens of meters)
4. Internal Waves (Meters to several tens of meters)
5. Surface Gravity Waves (Meters to less than two hundred meters)
6. Fronts (Tens to hundreds of meters)
7. Subinertial Frequency currents (Tens to hundreds of meters)

b. Horizontal

1. Surface gravity waves (Meters to hundreds of meters)
2. Internal waves (Tens to hundreds of meters)
3. Estuarine Plumes (Kilometers cross-plume axis to tens of kilometers down plume axis)
4. Fronts
 - a. Shelf Slope (Hundreds of meters cross axis and tens of kilometers to hundreds of kilometers along axis)
 - b. Gulf Stream Front (Tens of kilometers cross axis to hundreds of kilometers along axis)
5. Subinertial Frequency Synoptic Scale (Wind Driven Currents) Tens to a hundred kilometers cross-shelf and hundreds of kilometers alongshelf

TEMPORAL

1. Surface Gravity Waves (Seconds)
2. Internal Waves (Minutes to hours)
3. Bottom Boundary Layer Mixing (Minutes to tens of hours)
4. Surface Layer Mixing (Hours to days)
5. Estuarine Circulation (Hours to days)
6. Wind Driven Currents (Hours to days)
7. Shelf Slope Front (Hours to days)
8. Gulf Stream Front (Days to weeks to months)
9. Seasonal Cycles - all of the above
10. Interannual Cycles - all of the above

METHODS AND PLATFORMS

Cross-shelf arrays are needed to evaluate both the along and cross-shelf transport. The arrays extend from 5 meter depths out to 3000 meters. Moorings are required along the shelf break in the alongshore direction, to evaluate transport of shelf, shelf-slope and Gulf Stream waters and carbon into or out of the study region. Norfolk Canyon, north of the Cape Hatteras confluence will be instrumented as will Hatteras Canyon to determine the relative importance of such features in regions of different boundary current effects and shelf environments.

A mooring array was designed to answer questions relative to those physical processes, which are believed to affect the distribution of organic carbon within the system, and to measure directly sources and sinks of carbon either into or out of the system. Bottom tripods addressed benthic, bottom boundary layer processes. Meteorological buoys, each with an upperocean electromagnetic (E-M) current meter attached, would be used to quantify air- sea momenta and buoyancy exchanges in this meteorologically complex domain. Coastal wind and sealevel will be obtained from National Climate Data Center (NCDC) and the National Ocean Survey (NOS). Here the Gulf Stream effects component of the DOE-Atmospheric Radiation Monitoring (ARM)

program would be entirely complementary to the DOE-OMP Broadband. Acoustic Doppler Current Profilers (ADCPs) would be used throughout the array, particularly in high shear zones as centroids about which conventional taut wire moorings can be located. The bottom tripods, the ADCPs and the upper surface current meters, collectively constitute a suite of velocity measurements which essentially cover the entire water column. Salinity, temperature, pressure, fluorescence and particle concentration measurements will be made throughout the array using in-situ conductivity-temperature-pressure (CTD) sensors, fluorometers, transmissometers and turbidity sensors. Sediment trap devices can be placed on taut wire moorings beyond the shelfbreak below the euphotic zone, at mid-depths and near the bottom as required.

Satellite and aircraft imagery sea surface temperature and near surface optical depth (AVHRR), ocean color (SeaWifs), ocean topography (Topex), synthetic and real aperture radar (SAR, RAR), surface sea state and wind (SSM/I), surface chlorophyll (Lidar) will also be obtained either in real time egs: (AVHRR optical depth, SeaWifs) or directly from collaborating government agencies (National Aeronautics and Space Administration, i.e. NASA-Jet Propulsion Lab, ONR-Naval Research Lab, NASA Wallops). The real-time satellite (AVHRR, and SeaWifs) data will be used in a real time mode to guide shipboard surveys. Other satellite data will be utilized to identify Gulf Stream (GS) related features outside and inside the array, and to determine the impact of these features on the enclosed region through correlation with direct, contemporaneous measurements of velocity and particle concentration and flux.

STRENGTHS AND LIMITATIONS OF PROPOSED RESEARCH

The limitations in meeting the physical oceanographic goals of the OMP program in the MAB are a lack of state of the art mooring array equipment. L.J. Pietrafesa has designed an array that will meet the expressed needs of the bio-geo-chemists while allowing for volumetric, salt and heat balances. A capital outlay is required. The PI's presently in the program are sufficient.

STATUS OF RESEARCH

This 3rd year period of OMP funding involves a four month field program which is coordinated with the National Science Foundation CoOP field program to be conducted near Duck, NC, and a 2 month field effort coordinated with the NSF/National Oceanic and Atmospheric Administration/National Undersea Research Program field program to be conducted at the proposed Mobil Drill site to the northeast of Cape Hatteras. The NSF CoOP program will be conducted in water < 20 m deep, i.e. the inner shelf, while the NSF/NURP program will be at 850m, on the upper slope, a known organic carbon deposition site.

Keywords: sediment dynamics, benthic boundary layer, circulation, hydrography, fronts

**PRINCIPAL
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PROJECT TITLE

ROLE OF MARINE AGGREGATES IN CARBON EXPORT
FLUXES ON THE CAPE HATTERAS CONTINENTAL
MARGIN

AMOUNT OF FUNDING FY 1993: \$100 K

SUMMARY OF GOALS

- A. Quantify the size, abundance, POC content, flux, and in-situ sinking rates of marine aggregates ($> 0.5\text{mm}$) in the OMP study region, concentrating on the outer shelf and slope/rise.
- B. Quantify the depth-dependent flux of POC in the deep slope "carbon depocenter" region.
- C. Integrate the above data sets to determine the contribution by marine aggregates of variable size and sinking rates to POC fluxes and off-shelf carbon export.

SPATIAL AND TEMPORAL SAMPLING SCALES

- A. Seasonal (winter, spring, summer) cruise-conducted measurements of marine aggregate size, abundance, and in-situ sinking rates to be obtained along the 3 cross-shelf transect lines and (possibly) along the shelf-break between lines 1 and 2.

B. Deployment of time-series sediment traps placed at 500 m and 50 m above the bottom on the deep slope/rise moorings. Traps would be programmed to sample biweekly and would be recovered and redeployed every 6 months.

METHODS AND PLATFORMS

A. Cruise-conducted, ROV (or submersible)-based, seasonal in-situ marine aggregate measurements (size, sinking rates, etc.), and collections. Vehicle-based methods of aggregate surveys and collection have been successfully developed and tested using an ROV off of California from 1991-1993.

B. Time-series particulate mass and POC fluxes will be obtained using high-resolution time-series sediment traps (McLane Research Labs., Inc. MARK VI or VII traps) deployed on subsurface, taunt-line moorings. The P.I. has had many years of experience with these trap systems.

STRENGTHS AND LIMITATIONS OF PROPOSED RESEARCH

Strengths: Submersible vehicle-based aggregate measurements allow for the assessment of horizontal as well as vertical variability in various aggregate parameters; also provides the opportunity to collect marine aggregates *in-situ*. The time-series sediment traps represent the "state of the art" as well as the recognized standard method for obtaining high-resolution temporal data sets of POC fluxes.

Limitations: The relatively high cost of conducting vehicle-based studies (due to vehicle leasing and pilot fees) may limit the extent to which we will be able to provide in-situ aggregate data sets throughout the study region. Key sites and/or transects for the vehicle-based aggregate measurements may have to be selected depending upon where ship-board measurements of DOM, POM and $p\text{CO}_2$ are concentrated, in addition to obtaining aggregate data sets from near the trap mooring localities. The time-series sediment traps cannot be deployed in the shallow upper shelf region due to the potential biasing of the particulate sampling by relatively high horizontal velocities and resuspension activity in the shelf environment, as compared with the deep slope/rise.

STATUS OF RESEARCH

A new ROV-based instrumentation for measuring aggregate size, abundance, and in-situ sinking rates has been successfully developed and tested, with the final analyses to be completed by spring-summer, 1995. Time-series aggregate size and abundance data sets and in-situ

aggregate sinking rates in Monterey Bay (obtained with the ROV-mounted instrumentation), were presented at the Feb. '94 Ocean Sci. Mtg. in San Diego.

Keywords: aggregates, POC flux, sediment traps, submersibles, marine snow

**PRINCIPAL
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PROJECT TITLE

ASSIMILATION AND TRANSFER OF CARBON IN
OLIGOTROPHIC AND EUTROPHIC COASTAL WATER
COLUMNS

AMOUNT OF FUNDING FY 1993: \$267 K

SUMMARY OF GOALS

The overall objective of this program was to determine the amount of fixed carbon in the water column, its forms, its sources, and the rates and mechanisms of formation and removal. We addressed the hypothesis that a greater fraction of the carbon fixed over the shelf escapes respiration *in situ* and is available for export to the sediments and to the slope. This hypothesis was tested in Louisiana shelf waters of the central Gulf of Mexico. Our observations and conclusions would then be used to revise our hypothesis for subsequent testing in the waters off of Cape Hatteras.

In the future we plan to expand our study to address the first of these specific objectives with emphasis on determining P-I parameters and optical variability (e.g. spectral absorption and scattering) within the euphotic zone in the Cape Hatteras region with special reference to the absorption and attenuation of light in the 8 wavelength bands which will be utilized by SeaWiFS. In this way we can improve upon our ability to model primary production on similar temporal and spatial scales as those to be used by the FRR fluorometry studies and provide the larger spatial estimates of primary productivity associated with the 1 km and 4 km resolution of SeaWiFS-based production estimates.

SPATIAL AND TEMPORAL SAMPLING SCALES

In future studies off Cape Hatteras, we plan to measure primary production on at least two time scales. P-I parameters will be determined using 30 minute incubations. These incubations will be conducted with 5-10 ml samples obtained at sunrise, local noon and near sunset. We will

also conduct sunrise to sunrise, 24 hour incubations conducted in 1 or 4 L polycarbonate bottles using a light quantity, light quality and temperature controlled on-deck incubator system. These simulated *in situ* incubations will be conducted on the same day as the P-I studies so that the production measurements will be comparable. The P-I and 24 hour incubation studies will be conducted at discrete stations in coordination with other OMP projects. We will determine euphotic zone integrated primary production values (i.e. $\text{mg C m}^{-2} \text{ d}^{-1}$) with each technique. In conjunction with the incubations we will measure surface distributions and vertical profiles of chlorophyll *a* and take vertical profiles of photosynthetically available radiation (PAR) and measure daily incident PAR. Another routine measurement we plan to make at each production station and during surface water surveys will be spectral absorption and scattering of light, with special reference to those wavelength bands to be utilized by SeaWiFS. When the SeaStar satellite, which will carry SeaWiFS, is eventually launched, we will be in a position to use our understanding of the spatial variability in spectral attenuation and absorption to provide production estimates at both the 1 and 4 km resolution scales of SeaWiFS images.

METHODS AND PLATFORMS

We plan to utilize both simulated *in situ* ^{14}C incubation and 'Photosynthetron'-style photosynthesis-irradiance methods in order to measure primary productions. We will then employ various modeling techniques, based on distributions of chlorophyll *a* and incident irradiance fields, in order to extend our production estimates to larger spatial scales. Our bio-optical studies will be based upon use of various light measurement instruments (e.g. Biospherical Instruments PNF-300 and LiCor irradiance data loggers) and the Wet Labs AC-9 absorption and attenuation meter in order to examine those factors which impact the irradiance field in coastal water columns. Each of these approaches requires shipboard studies and deployments. Vessels with suitable deck space for setting up our incubation system (about 5' x 10') and for placement of a radioactive materials laboratory van would be required for us to complete our research objectives.

STRENGTHS AND LIMITATIONS OF PROPOSED RESEARCH

One of the strengths of our approach to making primary production measurements is that we would be able to provide independent determinations of C fluxes through the phytoplankton on time scales which are comparable to FRR based estimates as well as on daily scales. In addition we will be able to provide production estimates based on larger remote sensing spatial scales (e.g. at both the 1 and 4 km resolution scales). The accuracy of our production measurements will be dependent on a number of factors. The simulated *in situ* incubations will be subject to volume and containment considerations and the P-I related measurements will be only as robust as the models upon which they are based. Our studies to date have given us every indication that these two types of incubation techniques provide highly consistent and quite reasonable values of production. One question which has yet to be solved is whether or not the Wet Labs AC-9 can reasonably distinguish between the various seawater components which

either absorb or scatter light in shelf waters (e.g. Case II waters). We plan to continue to evaluate the performance of this instrument in an upcoming cruise to the waters off of Cape Hatteras.

STATUS OF RESEARCH

To date, we have completed 2 research cruises in the Gulf of Mexico shelf waters off of Louisiana. In addition, we will be participating on the upcoming R/V Gyre cruise to the Cape Hatteras study region (June 27-July 12, with Drs. P. Santschi, R. Jahnke, L. Benninger and I. Walsh).

The Gulf of Mexico work involved collaboration with Drs. R. Benner, M. Dagg and S. Strom. We explored the production, assimilation and fate of organic carbon in the Louisiana shelf waters of the northern Gulf of Mexico. We found out that recycling of carbon through both the micro- and macrozooplankton represented major losses of particulate organic carbon. Much of this carbon was transformed from particulate to dissolved organic forms. Bacterial production rates were reasonable relative the measured rates of primary production. One important finding was that the vertical export of dissolved organic carbon associated with sinking particles collected in free-floating sediment traps was approximately equal the export of POC. The export of DOC represents a major pathway for the loss of organic carbon from the photic zone in Gulf of Mexico shelf waters. Our Gulf of Mexico results were consistent with previous VERTEX findings for the North Pacific central gyre where we also found that DOC export was comparable to POC export.

Keywords: photosynthesis, primary production, satellites, POC flux

**PRINCIPAL
INVESTIGATOR(S)**

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PROJECT TITLE

CHARACTERIZATION OF MACROMOLECULAR
COMPONENTS OF PARTICULATE, COLLOIDAL AND
DISSOLVED ORGANIC MATTER AT THE SEDIMENT-
WATER INTERFACE. IMPLICATIONS FOR ORGANIC
MATTER DEGRADATION VERSUS PRESERVATION
DURING EARLY DIAGENESIS IN SHELF SEDIMENTS

AMOUNT OF FUNDING FY 1994: \$378 K

SUMMARY OF GOALS

The overall goals of our research as part of the OMP mission are the following:

- (i) To develop instrumental techniques for the quantitative analysis and characterization of complex polymeric constituents in various organic phases.
- (ii) To compile a comprehensive inventory of major biochemicals in particulate, colloidal and dissolved organic matter (POM, COM and DOM).
- (iii) To quantitatively assess the contribution of continentally derived organic carbon to margins.

SPATIAL AND TEMPORAL SAMPLING SCALES

We will propose two field efforts, one to examine internal cycling of DOM within the Hatteras study area, and a second survey study to monitor DOM (and POM) sources, accumulation and transformation during longshelf transport from Georges Bank to Hatteras.

Our Hatteras-based studies will be organized around compositional changes in DOM with changes in production and source terms, and on interfacing the biomarker approach with microbiological studies (¹³C labelling experiments) of DOM degradation/transformations. We will perform compositional characterization of DOM collected during four cruises throughout the course of the field program, in late summer (July/August), early winter (November/December), early spring (February/March) when river discharge is at its maximum, and late spring (March/April) when river discharge is less and local production is highest.

Depending on the size and location of the study cube, we envision taking samples at an inshore, midshelf, and offshore site along the north and south faces of the cube.

Our long shelf studies will largely follow the pattern of our recently completed spring cruise. We will survey the longshelf distribution by making 7 transects (approximate equal spacing) perpendicular to the shelf between Georges Bank and Hatteras, as well as one or more major estuaries (Hudson, Delaware, Chesapeake). Hydrocasts will be performed at 8-10 stations of 5-10 mile spacing per transect beginning at the 15 m contour and extending to the 1500-2000m contour. At each station we sample water at 5-25m depth intervals. We will propose 2 cruises in order to conduct these measurements in the spring (February/March) and again in the late summer/early fall (August/September).

METHODS AND PLATFORMS

For sampling, we use a deck-mounted large volume pumping system to filter seawater ($<0.2\mu\text{m}$) into ~200L barrels for ultrafiltration. DOM is concentrated to 1L by passing through 1KDalton or 10KDalton membranes, diafiltered, then frozen for return to the lab. We can process 2-4 samples per day. Tangential flow filtration (0.3 or $0.8\mu\text{m}$ membranes) of smaller volumes of seawater (4-20L) from Niskin Go-Flo Bottles is also performed for isolation of particulate matter (for mass spectral characterization). The latter samples can be processed within a few hours and therefore allow a more general survey type study, in which samples are collected at a spatial and temporal resolution comparable to many bulk measurements. Sample collection requires at least three (preferably four) people. For the longshore transport survey, we make POC, PON, HPLC pigment, nutrient, fluorescence, and DOC analyses on discrete samples. We also use underway fluorescence to monitor DOM and chlorophyll, and last spring coordinated our efforts with NASA P-3 overflights and satellite AVHRR and CZCS imagery. For the OMP field program, we would use our connections with NASA-GSFC to coordinate our efforts with SeaWiFS data. We are currently examining the potential of a fiber-optic DOM fluorescence profiler as well as a mooring-based sensor for DOM fluorescence, although it is unclear whether we will attempt to deploy these in the 1995-1996 time frame.

STRENGTHS AND LIMITATIONS OF PROPOSED RESEARCH

We are very keen on interfacing the longshelf survey with the detailed biogeochemical and physical oceanographic investigations at the Hatteras study site. On this year's spring cruise we observed large gradients in DOC between Georges Bank and Hatteras, and we suspect that estuaries play a major role in the export of DOC from the shelf. We do not believe that either approach will succeed in isolation. The focus on the Hatteras region makes sense from the viewpoint of understanding basic recycling mechanisms and there is a large and variable signal to be monitored. However, the study area is physically dynamic (as evidenced by AVHRR imagery and spatial DOC concentrations) and DOC is so strongly influenced by sources and inputs upshelf that some survey on a larger spatial scale is necessary. The importance of a

"whole-shelf" survey is also warranted in the light of the potentially small variations in bulk geochemical properties, such as $\delta^{13}\text{C}_{\text{TOC}}$ (due to dilution of discrete signatures). We hope to magnify these variations through molecular-level structural and isotopic measurements, however, in order to "calibrate" the observed compositional variations we need to fully characterize the magnitude and chemical signatures of the end-member source terms. For these purposes, sampling end-member inputs is essential.

STATUS OF RESEARCH

We have made substantial progress since the project's inception. The samples we have studied to date are derived from 2 successful field programs - a 5-day cruise to Georges Bank (April, 1993), and a 14-day cruise from Georges Bank to Cape Hatteras (April, 1994). Principal objectives of the first cruise were to test equipment, perform a methodology intercomparison for DOC sampling and measurements, collect DOC samples during a period of high phytoplankton productivity, and collect cores for macromolecular characterization and compound specific (biomarker) carbon isotope analyses.

Keywords: POC/DOC/COC, polymers, POM, DOM, mass spectrometer

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PROJECT TITLE

THE PRODUCTION OF COLLOIDS IN THE BENTHIC
BOUNDARY LAYER AND PARTICLE-PARTICLE
INTERACTIONS

AMOUNT OF FUNDING FY 1994: \$262 K

SUMMARY OF GOALS

1) Characterize the colloidal material extracted from 1kD, 3 kD and 10kD ultrafiltration cartridges by measuring concentrations of thorium (such as ^{234}Th , ^{232}Th , ^{230}Th) and carbon isotopes (such as $\delta^{13}\text{C}$, $\Delta^{14}\text{C}$), selected chemical biomarkers (such as plant pigments, lignin-phenols, and loliolides), trace elements (such as Al, Fe and Pb), and acid/base properties. 2) Investigate the relationships between chemical, biochemical (pigment and lignin-phenol biomarkers) and isotopic composition and residence times of colloidal organic carbon produced on the continental margin off Cape Hatteras. 3) Reconcile the apparent discrepancy between old apparent ^{14}C age of DOC and COC in the ocean and short turnover times of fractions of the DOC pool, using radiochemical and biochemical approaches. 4) Determine relationships between dissolved and particulate Th scavenging rate constants and particle or DOC concentrations along near-shore/off-shore gradients. 5) Determine relationships between water column fluxes and resuspension rates of COC and POC, dissolved and particulate scavenging rate constants of Th nuclides and the nature of colloids in the water column. 6) Determine the relationships between hydrodynamic parameters in the water column and sediment resuspension rates of colloidal organic carbon in controlled microcosm and/or flume experiments. 7) Using the geochemical

parameters described under 1-5, and the hydrodynamic parameters determined by physical oceanographic programs, calculate the fluxes of colloidal organic carbon from the continental margin to the open ocean, in particular for the colloids in the benthic nepheloid layer.

SPATIAL AND TEMPORAL SAMPLING SCALES

1) Near-shore/off-shore transects, combined with 2 along-shore transects (with sampling as a function of distance and as a function of depth) for sampling of dissolved, colloidal and particulate radioactive and stable isotopes, colloids, pigments, lignin-phenols, DOC, SPM. 2) 200-800 m water depth: Sediment trap and Alabaster Lander deployment.

METHODS AND PLATFORMS

During field expeditions, hydrographic surveys using CTD, fluorometry and transmissometry casts are carried out. Water samples are collected for radiochemical, chemical and biochemical analyses, and samples are analyzed in the lab; in addition, lab experiments to study the resuspension of colloids are continued. Methods include the following:

MIPS: Multiple In-situ Pumping System for obtaining vertical profiles of radioisotopes.

Ultrafiltration: Large-volume Cross-flow Ultra filtration, which allows us to obtain 100-1000 mg freeze-dried colloids from 200-1000 liters of sea water, after diafiltration of the concentrate.

Sediment traps: Large-area, swimmer-repellent multi-sampling sediment traps will be used for the purpose of collecting enough sinking particulate organic carbon material to be deployed for ≥ 4 days.

Alabaster Lander for Benthic Boundary Layer measurements: u^* and D/z or bottom stress by gypsum dissolution, deployed for 0.5-2 days.

Radioisotope measurements: ^{234}Th : Analysis by gamma-counting. ^{232}Th : Analysis by Mass Spectrometric methods. ^{230}Th : Analysis by Secondary Ion Mass Spectrometric methods (SIMS).

Elemental analysis: by ICP-MS and standard methods (for C, N).

DOC: Our methods have been intercalibrated with those employed in R. Benner's laboratory, with satisfactory agreement to within a few %.

POC&SPM from suspended matter: Laboratory of J. Brooks for POC.

$\delta^{13}\text{C}$: A working relationship has been established with the Lab of L. Cifuentes.

Nutrients, oxygen: On shipboard from discrete water samples, using Technicon AA-II autoanalyzer.

Total Pigments: In-situ fluorometry. Plant pigment compounds (carotenoids and chlorophylls) are analyzed using reverse-phase HPLC.

Lignin-phenols are analyzed using capillary GC. Loliolides are analyzed using normal phase HPLC.

$\Delta^{14}\text{C}$: Colloids are processed for ^{14}C targets by S. Trumbore, under a subcontract, and analyzed by J. Southon, Lawrence Livermore National Laboratory.

Vertical profiles of Th isotopes (^{234}Th , ^{232}Th , ^{230}Th and ^{228}Th), oxygen, nutrients, fluorometry and

transmissometry are used to concurrently define scavenging regimes, coagulation rates, and remineralization rates.

STRENGTHS AND LIMITATIONS OF PROPOSED RESEARCH

Strengths

- Collection and measurement of DOC/COC in field: Collection of up to 40% of DOC is possible by crossflow ultrafiltration and 1kD cut-off ultrafilters. Colloids are size fractionated using 1kD, 3kD and 10kD ultrafilters.
- Radiochemistry in water column: One can obtain important rate parameters relevant to organic carbon cycling, including rate constants for scavenging, particle cycling and colloidal turnover.
- Isotopic and elemental composition of colloids from field experiments: Modern ^{14}C in $\geq 10\text{kD}$ fraction. Relationships between ^{234}Th -derived scavenging and particle cycling rate constants (λ_s , λ_p) and fraction of ^{234}Th associated with colloids, COC/DOC fraction, and SPM concentrations.
- Biomarkers from field experiments: Potential of using pigment biomarkers in colloids as dating tools.
- Benthic resuspension rates of colloids by measurements in lab and field: Development of a colloid flux model including COM/sediment partitioning and the development of shear stresses at the benthic boundary layer. Measurement of COM fluxes from or to sediments as a function of applied shear stress using box core material and a stirred benthic chamber.

Weaknesses and potential problems:

- Collection and measurement of DOC/COC in field experiments: Long collection times (8-20 hours, but not affecting station time for surface samples; subsurface samples require about 2 hours per depth), relatively large concentration factors are necessary to isolate colloids from 200-1000 L of seawater. Calibration experiments necessary for evaluating potential for artifacts and blanks (filtration, ultrafiltration) in lab and field. Experiments are currently carried out in the lab, and more will be carried out in the intercomparison experiments ("colloids cook-out") organized by Ken Buesseler in the field. More experiments have been proposed to NSF in a separate proposal.
- Radiochemistry in water column: Relatively large requirements on wire time (2 hours/depth).
- Isotopic and elemental composition of colloids from field experiments: Calibration experiments are necessary to evaluate potential for artifacts and blanks. Several experiments have been carried out, and more are planned.
- Biomarkers from field from field experiments: Black plastic is employed to reduce potential for photochemical degradation, cooling coils to reduce breakdown due to heat during

ultrafiltration, calibration experiments test cell lysis and breakdown during filtration and ultrafiltration procedures. Some of these have been carried out, some more are planned.

- Benthic resuspension rates of colloids by measurements in lab and field: Long time scales of these experiments, if one wants rigorous approach.

STATUS OF RESEARCH

During this funding period, three sampling expeditions to the Gulf of Mexico, and one cruise to the Cape Hatteras region were undertaken. Another one is planned for June 27-July 13, 1994, and a colloids sampling intercomparison experiment ("colloids cookout"), organized by Ken Buesseler, is planned for August 1994.

During the past two years, we have acquired a unique combination of capabilities and techniques which enabled us to tackle the colloidal puzzle head-on: not only can we extract large volumes of seawater for ^{234}Th and colloid analysis [described in Baskaran et al., 1992, 1993a,b; Guo et al., 1994a], we can also isolate and characterize large quantities (100-1000 mg) of colloidal matter [Guo et al., 1994a]. Furthermore, we have also established low blanks and excellent mass balances for ultra filtration of DOC, ^{14}C and ^{234}Th (as described above). ^{230}Th and ^{232}Th blanks are satisfactory for MIPS samples, but not yet for all ultrafiltration samples (see above). We also routinely determine the weights of SPM and colloids sampled for radionuclide analyses, which are used in some of our models. We have organized and participated in 3 cruises to the Gulf of Mexico, and one to the Cape Hatteras area.

Keywords: colloids, isotopes, biomarkers, residence times, thorium, resuspension, benthic boundary layer

**PRINCIPAL
INVESTIGATOR(S)**

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PROJECT TITLE

CONTROLS ON MARINE CARBON FLUXES VIA
PHYTOPLANKTON-MICROZOOPLANKTON
INTERACTIONS IN CONTINENTAL SHELF WATER

AMOUNT OF FUNDING FY 1994: \$77 K

SUMMARY OF GOALS

The goals of this project were to develop new approaches to assaying protistan grazing on phytoplankton (and on bacteria) in shelf waters. Current approaches for determining in situ microzooplankton grazing rates are in general cumbersome, requiring extensive sample manipulation and fairly long incubation of living organisms. Such methods preclude high-resolution sampling of protist grazing rates. We have spent much of our time on this grant developing enzyme-based assays which will allow increased sampling resolution for protist grazing on bacterial and phytoplankton prey in situ. The Digestive Enzyme Assay (DEA) approach is based on determining the rate of cleavage of a fluorochrome, methylumbelliferyl (MUF) from surrogate substrates at acid (pH 4.5) pH, the pH of protist food vacuoles. The DEA method permits a 'snapshot' of protist digestive enzyme activity and is an in vitro, rather than an in vivo, technique, thus avoiding artifacts associated with manipulation and incubation of live protists.

SPATIAL AND TEMPORAL SAMPLING SCALES

The strategy for sampling of protist grazing rates in the OMP program is to obtain data over short time periods within distinct seasons, i.e. intensive sampling during individual cruises. We plan to determine distribution of protistan grazing activity (herbivory and bacterivory) with depth, from inner to outer shelf regions, and between separate transects.

METHODS AND PLATFORMS

Our sampling program will require collection of water samples during scheduled cruises in conjunction with the sampling programs of the other biologists. Methods of assessing protist grazing will be: short-term uptake of fluorescently labeled bacteria and acid lysozyme activity

for bacterivory, and acid β -galactosidase activity for herbivory. Samples will also be preserved for later microscopic inspection of the protistan community in the nanoplankton and microplankton size ranges.

STRENGTHS AND LIMITATIONS OF PROPOSED RESEARCH

Protistan grazing on bacteria and on phytoplankton is a major process in marine food webs. Thus, in order to properly evaluate pathways of carbon flow in marine systems, it is essential to have a good understanding of the trophic activities of heterotrophic protists. Unfortunately, existing assays for protist grazing are so cumbersome to carry out that not very many measurements can be done within short time periods (days). The DEA approach that we are planning to use will be the first method which allows high-resolution sampling for protist grazing, so that vertical and horizontal variability in grazing activity can be assessed. The weakness of the method is that this is a new approach, and we are just now working out a calibration curve to allow quantification of herbivory (chl-a mortality) based on measured in situ β -galactosidase activity.

STATUS OF RESEARCH

Under the auspices of the OMP, the first application of the DEA approach was the acid lysozyme method developed by our post-doctoral colleague, Dr. Juan Gonzalez (1993, MEPS 100:197-206). Rate of cleavage of MUF from a fluorochrome linked substrate analogue of peptidoglycan, a major structural compound in bacterial cell walls, was calibrated as a quantitative indicator of bacterivory via rates of uptake of FLB. We are now evaluating measurement of β -galactosidase-like activity at acid pH as an indicator of herbivory. All phototrophic organisms, including cyanobacteria, have sulfo-lipid compounds associated with their chloroplasts (thylakoid membranes) that can be cleaved by the enzyme β -galactosidase. Thus, herbivorous protists should elaborate digestive enzymes for cleaving the sulfo-lipids of phototrophs, but which also would be able to cleave MUF from the substrate MUF-galactoside. Preliminary results using this substrate are promising. Herbivorous protists in lab culture and in natural seawater assemblages have high β -galactosidase-like activity at acid pH, comparable in magnitude to β -glucosidase activity. Bacterivorous protists have negligible β -galactosidase-like activity. The challenge now is to calibrate measured β -galactosidase activity by simultaneous determination of actual chlorophyll loss due to grazing. We are attempting to do this via comparison of MUF- β -galactosidase-like activity with chlorophyll grazing loss estimated via dilution experiments using coastal seawater.

We plan to use DEA methods for both bacterivory and herbivory on the upcoming cruise in June. We will carry out on-board FLB-uptake experiments to calibrate the acid lysozyme methods, and will use a laboratory-based calibration factor to convert acid β -galactosidase activities to phytoplankton grazing rates.

The data obtained on the June cruise, combined with data collected on the DOE cruise last May, by ourselves (bacterivory via FLB-uptake) and others, should give a fairly detailed picture of the abundance, biomass distribution, and grazing activities of the heterotrophic protist assemblage in the context of the biomass standing stocks and productivities of their bacterial and phytoplankton prey. The June cruise will be the first time that high-resolution sampling of the grazing activity as well as abundance of protists will be made.

Keywords: microzooplankton, herbivory, bacterivory, enzyme assays

**PRINCIPAL
INVESTIGATOR(S)**

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PROJECT TITLE

CONTROLS ON MARINE CARBON FLUXES VIA
PHYTOPLANKTON-MICROZOOPLANKTON
INTERACTIONS IN CONTINENTAL SHELF WATER

AMOUNT OF FUNDING FY 1994: \$78 K

SUMMARY OF GOALS

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Keywords: microzooplankton, herbivory, bacterivory, enzyme assays

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PROJECT TITLE

THE ROLES OF ADVECTION AND IN SITU GROWTH IN
DETERMINING THE DYNAMICS OF CONTINENTAL
SHELF ZOOPLANKTON: HIGH FREQUENCY
MEASUREMENTS OF ZOOPLANKTON BIOMASS
COUPLED WITH MEASUREMENTS OF SECONDARY
PRODUCTIVITY IN THE MIDDLE ATLANTIC BIGHT

AMOUNT OF FUNDING FY 1994: \$81 K

SUMMARY OF GOALS

In trying to quantify the extent to which physical and biological forcing are responsible for observed distributions of zooplankton, in a way that helps us understand and predict how systems work, we need to be able to extrapolate biological forcing (variables associated with population growth), usually observed on small to mesoscales, to larger scales. And we need to know in general whether physical forcing has a positive or negative feedback to the biological system. Any extrapolation is dependent on larger scale observations, such as an assessment of biomass by the acoustic Doppler current profiler, which have been impossible to make until recently. We will build on our knowledge from our earlier studies and investigate the "biomass field", using the acoustic Doppler current profiler, and the processes that can be deduced from combining the biomass field with measurements of growth (egg production), with measurements of grazing rates, and with formalized, algorithm-like treatments of other processes such as respiration.

To establish quantitatively the role of larger zooplankton in the cycling of carbon on the continental shelf of the northeastern United States by measuring biomass using five acoustic Doppler current profilers deployed as a box, by measuring biomass using towed MOCNESS nets, and by measuring growth and grazing rates using ship-board incubation experiments. To separate and understand the roles of advection and in situ growth in determining the biomass patterns observed and their impact on the carbon cycle.

SPATIAL AND TEMPORAL SAMPLING SCALES

Spatial sampling scale: mesoscale

Temporal sampling scale: occasional daily burst sampling nested within the seasonal and annual cycles.

METHODS AND PLATFORMS

Methods: moored acoustic Doppler current profilers (5), ship-board acoustic Doppler current profilers to map biomass, MOCNESS tows to "calibrate" the acoustic Doppler current profiler, live tows for organisms for measuring rates in on-deck incubation experiments.

Platform: moorings and research vessels.

STRENGTHS AND LIMITATIONS OF PROPOSED RESEARCH

Strength: proven and sensible approach to doing biological oceanography.

Weakness: requires money.

STATUS OF RESEARCH

Ongoing; the work envisioned for the Ocean Margin Program is an extension of prior work in the Gulf Stream, California Current and Middle Atlantic Bight.

Keywords: metazooplankton, biomass, grazing, ADCP

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PROJECT TITLE MECHANISMS OF DISSOLVED ORGANIC CARBON
CYCLING IN AN OCEAN MARGIN

AMOUNT OF FUNDING FY 1994: \$114 K

SUMMARY OF GOALS

Dissolved organic carbon (DOC) is the largest organic carbon reservoir in the ocean. It is important to understand the processes by which DOC is cycled because changes in the oceanic DOC pool could affect atmospheric carbon dioxide concentrations. Phytoplankton are the dominant source of marine DOC, but the mechanisms and pathways of DOC formation are poorly understood. This study will focus on the production of DOC during herbivorous grazing on both eucaryotic and procaryotic phytoplankton. Herbivory is likely the major pathway for the transformation of organic matter from particulate to dissolved form by grazers. A combination of laboratory and field experiments will be used to determine the following:

- the relative importance of herbivory for DOC production,
- the rates and chemical characteristics of DOC production by natural communities and particular species of phytoplankton, micro- and mesozooplankton,
- the effect of phyto- and zooplankton species on the quantity, molecular size, and composition of released DOC,
- the degree of coupling between DOC production and bacterial utilization.

SPATIAL AND TEMPORAL SAMPLING SCALES

We propose characterizing herbivorous grazing and DOC release at inner, mid and outer shelf locations within the framework of process-oriented "transformation cruises." The experimental timescales of the grazing experiments are well-matched with the free-floating

sediment trap deployments of ~2 days. These experiments should be conducted within the vicinity of the cluster mooring sites. These processes will be measured during seasonal cruises to the study region off Cape Hatteras.

METHODS AND PLATFORMS

Laboratory experiments will be conducted in mesocosms with phytoplankton and zooplankton species representative of the major taxa known to play a major role in coastal marine systems. Grazing by microzooplankton and concomitant DOC production will be estimated using the dilution technique of Landry and Hassett (1982). Bacteria will be DAPI-stained and enumerated using epifluorescence microscopy (Porter and Feig, 1980), and phytoplankton and protozoa will be counted using either epifluorescence or inverted microscopy (Strom and Buskey, 1993). Bacterial production will be estimated from rates of protein synthesis as measured by the incorporation of ³H-leucine (Kirchman et al., 1985). Tangential-flow ultrafiltration will be used for separating DOC into various molecular sizes for chemical characterization (Benner, 1991). Chemical characterizations of samples will include measurements of DOC by high-temperature combustion (Benner and Strom, 1993), dissolved carbohydrates by spectrophotometry (Pakulski and Benner, 1992), neutral sugars by HPLC (Mopper et al., 1992), dissolved amines by HPLC (Gardner and St. John, 1991), and pigments by HPLC (Wright et al., 1991).

STRENGTHS AND LIMITATIONS OF PROPOSED RESEARCH

The proposed experiments will help elucidate the fundamental mechanisms and major pathways of carbon flow between the autotrophic and heterotrophic components of marine food webs. Knowledge of mechanisms and pathways of carbon flow is essential for determining environmental factors that regulate the ultimate fate of carbon in the ocean. The proposed grazing experiments require 1 to 2 days to complete so spatial coverage during cruises will be limited and we must assume that the measured rates of DOC production and consumption are representative for the general study region.

STATUS OF RESEARCH

This is a new project that will start on September 1, 1994.

Keywords: DOC, zooplankton, grazing, phytoplankton

**PRINCIPAL
INVESTIGATOR(S)**

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PROJECT TITLE

REGULATION OF PHOTOSYNTHETIC CARBON FIXATION
ON THE OCEAN MARGINS

AMOUNT OF FUNDING FY 1994: \$76 K

SUMMARY OF GOALS

The goals are to 1) understand regulation of ribulose biphosphate carboxylase (RubisCO) in phytoplankton cultures in response to light regime 2) determine regulation of RubisCO in response to light during nutrient limitation in these cultures 3) to determine mechanisms of RubisCO regulation in natural populations of phytoplankton on the ocean margins in the Gulf of Mexico and 4) to measure regulation of RubisCO in phytoplankton of the Hatteras System. Two goals are laboratory-based, and two are ship-based.

During the first year, the Tabita lab is primarily responsible for objectives 1 and 2 of the overall project (see above). Specifically, we have initiated experiments to probe the regulation of RubisCO in response to different light and nutrient limitation conditions in representative marine cyanobacteria and diatoms. These studies have been performed to understand the factors that control RubisCO gene transcription and RubisCO activity, and to relate these molecular perturbations to changes in the marine environment that influence overall CO₂ and carbon metabolism.

SPATIAL AND TEMPORAL SAMPLING SCALES

Participation in DOE cruises is highly desired. We would be interested in spatial sampling across the shelf in conjunction with the spring bloom at least 4 stations along a transect (for vertical profiles). During a second cruise, diel regulation and regulation under various light regimes is required for us to complete our DOE mission. These studies last 24-48 hr/diel with sampling every 4 hr.

METHODS AND PLATFORMS

Laboratory Studies. To pave the way for studies with organisms that are more difficult to culture and extract, we have begun our investigations with marine cyanobacterial strains with which we have some experience. *Anabaena* sp. strain CA is a marine nitrogen fixing organisms that we isolated, along with several other strains, 17 years ago. It is representative of a group of marine cyanobacteria found on the ocean margins and it grows well in the lab. We have worked out all the basic techniques for measuring RubisCO transcripts from this organism under both light and nutrient limitation. These studies resulted in the significant observation that the RubisCO genes (*rbcLrbcS*) and the gene (*rca*) that specifies an enzyme that modulates RubisCO activity *in vivo*, namely RubisCO activase, are independently regulated at the level of transcription. These studies are relevant to understanding the ability of RubisCO to fix CO₂ which drives the ecosystems of the ocean margins.

For the study of RubisCO regulation, four types of biological measurements are required: 1) measurement of transcriptional regulation, by extraction and quantitation of *rbcL* mRNA 2) measurement of RubisCO enzyme activity in extracts of cells 3) determination of the amount of RubisCO protein, determined immunologically and 4) determination of whole cell carbon fixation. These methods provide closure on all mechanisms of regulation for this enzyme. Additionally, we will amplify, clone, and sequence some *rbcL* genes from natural populations of phytoplankton and from specific cultures provided to us by collaborators and of our own isolation.

For the studies described herein, approximately 20x20' deck space is required. A vessel with a CTD with a fluorometer probe, light meter, and rosette sampler is required. In terms of shipboard lab facilities, 2 fume hoods are required, one for mRNA extraction, one for filtration of samples treated with DEPC (diethyl pyrocarbonate). Adequate space for radioisotope work is also required. Deck space for incubators is also needed. For these studies to be performed in conjunction with other components of the DOE OMP, a reasonably large vessel will be required.

STRENGTHS AND LIMITATIONS OF PROPOSED RESEARCH

Strengths. Clearly one of the greatest strength of this project is the capability of measuring phytoplankton gene expression by mRNA isolation and quantitation. A second strength is the amplification of *rbcL* genes for cloning and sequencing, which will enable determination of the types of organisms responsible for oceanic carbon fixation.

Limitations. A potential limitation of the project deals with uncertainty on the appropriateness of our cyanobacterial gene probe to detect oceanic picoplankton *rbcL* genes. Although we have had a high degree of success using the *A. nidulans* (= *Synechococcus* PCC6301) probe, some recent studies in the Tabita lab indicate that it only weakly hybridizes with *Synechococcus* WH7803 (although this organism may not be representative of oceanic

picocyanobacteria). Our preliminary studies on the Pacific *Prochlorococcus* by sequence analysis suggests that it would hybridize well with our *A. nidulans* probe. We are examining production of probes from oceanic picocyanobacteria we have in culture to mitigate this potential limitation, as well as using our diatom probe to get the "golden" clade of phytoplankton.

A second concern is how to do diel studies. Current studies with deck top incubators suggest that conditions in these deteriorated in as few as 12 hr, which make studies of any duration difficult. The alternative, tracking a water mass by a drogued buoy, does not enable manipulation of light regimes and does not preclude vertical migration of the phytoplankton population out of the zone of sampling.

STATUS OF RESEARCH

As mentioned above, we are making good progress in lab studies with phytoplankton isolates and in field studies of natural populations in terms of the regulation of RubisCO. In October, we participated in laboratory studies on the regulation of *rbcL* using Pacific *Prochlorococcus* cultures in the lab of Dr. Lisa Campbell, University of Hawaii. The regulation of *rbcL* was studied through light and dark cycles of a 52 hr diel study. The results of these experiments are forthcoming as the data is being processed. We participated in a cruise in September to study regulation of RubisCO in vertical profiles and during a diel experiment. We have planned a cruise for August, funded by a NSF project. This cruise will examine regulation of RubisCO in the field, as well as look for transcriptional regulation throughout the water column. A third cruise is scheduled off the Hatteras area in summer of 1995, again funded by NSF.

A major publication based on this work will be submitted to a leading journal by the time this report is received. Most important, however, is the fact that our experience with *Anabaena* has provided us with the necessary expertise to tackle aspects of regulation in open oceanic strains, organisms which are much more difficult to cultivate. To assist in these efforts, we have isolated an oceanic unicellular strain from the Gulf of Mexico, obtained on a cruise in September of 1993. This strain grows fairly rapidly (unusual for such organisms) both on liquid and solid marine growth media. The latter point is particularly important for any future studies on the genetic regulation of CO₂ fixation in marine cyanobacteria. Most interesting is the fact that this new isolate may be capable of fixing nitrogen. If confirmed, this will be an exciting observation, for it may mean that such organisms contribute to the nitrogen budget of the oceanic environment as well.

Keywords: photosynthesis, ribulose biphosphate carboxylase, nutrient limitation

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PROJECT TITLE

MOORED INSTRUMENT FOR TIME-SERIES STUDIES OF
PRIMARY PRODUCTION AND OTHER MICROBIAL RATE
PROCESSES

AMOUNT OF FUNDING FY 1993: \$132 K

SUMMARY OF GOALS

Our laboratory has endeavored to reduce the temporal disparity between key biological measurements, and correlative high resolution physical and biophysical measurements, as well as to provide a means for investigating underlying physiological response of microorganisms to changes in the environment. Under recent DOE funding we have built a Time Series - Submersible Incubation Device (TS-SID), a mooring-compatible, automated instrument for performing multiple in situ microbial incubation experiments. Incubations are performed under conditions that accurately simulate the environment and requires no involvement of the investigator other than the analysis of samples at the end of the deployment. Because most of the labor intensive manipulations have been automated in this instrument it is possible to greatly increase the frequency of measurement in biological time series studies (up to several measurements per day) for directly quantifying the effects of seasonal or episodic physical phenomena upon biological activities such as phytoplankton primary production.

SPATIAL AND TEMPORAL SAMPLING SCALES

The temporal scale of measurements will range from 4 measurements per day to measurements every several days. The exact duration of a deployment will depend upon frequency of sampling. Instruments possess 6 mo. longevity but will likely be finished with measurements before that. Ideal deployment period is 3 mo. Spatial and depth dependent scale will be a function of the number of instruments available (probably 3-4).

METHODS AND PLATFORMS

The TS-SID's operate completely autonomously and possess a mooring-strength frame, permitting the instruments to be incorporated directly into the mooring line in series with other instrumentation (cage dimensions housing instrument components, 27" x 27" x 41", not including apex to attachment points at either end; total length from attachment point to attachment point, 82"). During ship-based cruises one or more of the TS-SID's may be deployed on free-drifting surface moorings. The moorings are tracked by radar, radio beacon and light flasher, and would require a cruise where the ship predominately stays within a region. Because of risk of instrument loss, deployments would not be compatible with survey cruises where time at a given station is short and the ship travels large distances. Ship laboratory space would be necessary for recycling of the instruments and C-14 sample processing.

STRENGTHS AND LIMITATIONS OF PROPOSED RESEARCH

Strengths: 1) Autonomous performance of in situ tracer incubation experiments, physiological studies possible. 2) A single instrument can be used for a wide variety of studies, depending upon tracers implemented. 3) Temporal resolution of incubations (e.g., primary production) usefully on par with high temporal resolution physical and bio-optical time series measurements. 4) The instruments may be deployed for relatively long periods. Biofouling control measures have been incorporated into the instrument so that the internal and external surfaces of the incubation chamber may be acid cleaned and kept free of light-occluding material. 5) The instruments are modular, size can be modified as a function of the number of incubations to be performed per deployment. For example, a version of the instrument to be deployed during cruises would be smaller than the moored instrument as biofouling control would not be required and approximately one-half the number of subsamples would be necessary. The instrument would be retrieved at appropriate intervals and recycled to preserve high temporal resolution.

Limitations: 1) Spatial and depth-dependent resolution a function of the number of instruments available. 2) A finite number of subsamples can be taken (93 pairs), hence, temporal resolution and deployment longevity are inverse functions of one another, and both variables are decreased if the number of subsamples per incubation are increased (i.e., time course incubations).

STATUS OF RESEARCH

We have built a Time Series - Submersible Incubation Device (TS-SID), a mooring-compatible, automated instrument for performing multiple in situ microbial incubation experiments. Each incubation involves a cleaning cycle, procurement of a 400 ml sample at depth with simultaneous introduction of tracer, and the preservation of up to four pairs of subsamples during incubations of user-determined length. The device is capable of conducting 47 in situ end point incubations (t_0 , t_1 ; i.e., zero time, time 1) or 31 three-point time course incubations (t_0 , t_1 , t_2) at user determined intervals. During each deployment a total of 93

subsample pairs (186 total) are preserved for subsequent analysis. If background (t_0) activities are sufficiently low, it is possible to make t_0 measurements at less frequent intervals, thereby increasing the total number of possible incubations. For example, if t_0 measurements were made every 5th incubation the total number of possible incubations increases to 75 (t_0 activities interpolated between each 5th incubation where the measurement is made). Initial emphasis has been placed upon C-14 measurements of phytoplankton primary production, but the device is applicable to any tracer study requiring incubation.

In the interdisciplinary field studies, we would perform the following measurements.

- 1) Deployment of TS-SID's (at least 3 units, hopefully) for moored high temporal resolution time series measurement of C-14 primary production. Instrument location will be coordinated with the along-shelf and across-shelf OMP mooring program so that the TS-SID data can be integrated with ongoing time series measurements of water column physical properties, nutrient dynamics and bio-optical properties, in particular fast repetition rate (FRR) fluorometry. During part or all of the deployment period the TS-SID's will be configured to measure C-14 primary production in multiple short term incubations (up to 4 incubations per day, 2-3 hr per incubation) to provide a measure closely approximating gross photosynthesis throughout the light period.

- 2) Implementation of the TS-SID's in a longer term incubation mode where measures of the intracellular distribution of C14 tracer will be implemented for estimating phytoplankton respiration in the light and dark, and for providing more accurate estimates of excreted organic carbon (EOC). The overall approach will take advantage of the instrument's ability to obtain time course subsamples during each incubation in a manner that will allow subsequent subcellular fractionation of samples for quantifying the dynamics of the flow of tracer into each of the major classes of biopolymers, low molecular weight metabolites and excretion products. This information will relate to the overall physiological state of the population (e.g., dynamic growth vs. senescence), and under the correct conditions can provide information on respiratory carbon loss and excretion.

Keywords: primary production, submersible remote incubator

**PRINCIPAL
INVESTIGATOR(S)**

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PROJECT TITLE **FOOD CHAIN DYNAMICS**

AMOUNT OF FUNDING FY 1994: \$194 K

SUMMARY OF GOALS

A. Provide a basic description of the inorganic carbon, oxygen and nutrient distributions in the OMP Study Area. Goal is to contribute data for validation of a seasonal, shelf-wide model of inorganic carbon cycling.

B. Determine the feasibility of using multiple correlations between inorganic carbon and/or nutrients with physical/chemical parameters that can be measured in situ from moorings and/or TOYO/SeaSoar systems in order to estimate high frequency variability and fluxes of key biogeochemical species within the OMP study area. One goal is to determine whether dense mooring arrays and high-frequency (e.g. days or less) measurements can be used to infer carbon transformations from measurements of the local convergence or divergence of inorganic carbon and other nutrients based on proxy measurements. Another is to utilize TOYO/SeaSoar data in order to provide robust estimates of the inventories of oxygen and inorganic carbon within a limited area in order to estimate low-frequency (e.g. seasonal) temporal inventory changes and hence infer net carbon transformations.

C. Support the calibration and implementation of a moored measurement program for dissolved gases: particularly oxygen, nitrogen and CO₂. Goal is to support and validate measurements of the Moored Sensing Systems program (C. Wirick, PI).

SPATIAL AND TEMPORAL SAMPLING SCALES

Alongshore Survey: A minimum of four alongshore dissolved inorganic carbon surveys of the entire Mid-Atlantic Bight should be conducted. These need not necessarily be conducted in a single year; however, seasonal coverage is important. At least seven cross-shelf transects should be occupied from Cape Cod to Cape Hatteras with >8 stations per transect, extending from the nearshore to the 1500m isobath. Samples should be collected every 5-10m in the vertical within

the upper 100m, with decreased density at greater depths. Sampling should extend as far as the oxygen minimum, which lies at ~200m over the slope.

Mooring Study/Support: At least three transects within the OMP study area will be occupied regularly with measurements of oxygen, dissolved inorganic carbon and nutrients. Currently there are 'standard transects' in the Cape Hatteras area at 36.5 °N, 36 °N and 35.5 °N. Station spacing is ~5km, with vertical sampling density of order 5m over the shelf, and slightly less in deeper water.

METHODS AND PLATFORMS

Platforms: Research vessels will be the primary platform given the space, power and personnel requirements of the analytical instrumentation required for high-accuracy measurements.

Methods: At least two of the inorganic carbon system parameters (CT, Titration Alkalinity, pCO₂, pH) will be measured on discrete water samples in order to characterize the inorganic carbon speciation and allow separation of the effects of metal carbonate vs. organic carbon changes on the total dissolved inorganic carbon concentration. We are developing a high accuracy, dye-based pH measurement for incorporation into BNL's automated SOMMA systems. This may be the most cost-effective and space- and labor-saving approach to the CO₂ system for the OMP program, as it requires only one sample and one analytical instrument. The BNL SOMMAs are also configured to measure salinity to an accuracy of ~0.01, which may be adequate for OMP purposes. In the interim, we will make potentiometric measurements of total alkalinity and/or discrete measurements of pCO₂ (GC/FID), in addition to the SOMMA's CT analysis.

Oxygen will be measured to WOCE accuracy guidelines by Winkler titration. Nutrients (nitrate, phosphate, silicate, nitrite, ammonium) will be measured at sea whenever possible using a Technicon Autoanalyzer system and standard methods. BNL has a long history of making nutrient measurements in the Mid-Atlantic Bight, and overall accuracy is close to WOCE guidelines.

Note we are not planning to make underway measurements of pCO₂. We would like to continue collaboration with Drs. Chipman and Takahashi (LDEO), who have extensive experience of underway measurement systems and who have already deployed such systems on several OMP cruises. We have found that collaboration and cooperation between the LDEO 'underway' and BNL 'discrete' programs have been effective to date.

STRENGTHS AND LIMITATIONS OF PROPOSED RESEARCH

The scientific goals outlined above are readily achievable assuming suitable resources (e.g. ship-time, personnel), and effective coordination of sampling opportunities within the OMP. BNL

has state-of-the-art instrumentation and techniques available for the parameters listed, although the aging Autoanalyzer may require replacement during the OMP study.

The main limitations to achieving the goals outlined above stem from the need to field up to four separate measurements systems on each OMP cruise. While the inorganic carbon, nutrient and oxygen measurements are central to the achievement of OMP goals, it has not been possible to measure all parameters at sea on any OMP cruise to date. We have been forced to take the less- desirable approach of collecting samples for subsequent shore- based analyses, which always entails a risk of degraded data quality or even data loss (e.g. ammonium measurements on frozen samples are suspect; samples may get thawed or damaged in transit from the ship). In addition, the flexibility to modify sampling schemes based on observed features is lost. This has been necessary due to a lack of berths and/or laboratory space on the research vessels; however, we are also effectively limited by the number of trained analysts we can pay to go to sea and process data.

STATUS OF RESEARCH

By the end of June 1994, our team will have participated in four OMP cruises. Sampling was as follows:

OMP-93 (Gyre G-6-93) 'Biology' Spring cruise. CT, pCO₂ (discrete), oxygen, nutrients (frozen samples). Three highly-detailed transects in Cape Hatteras vicinity were sampled. The LDEO team assisted us with the CO₂ analyses, as well as operating their underway pCO₂ system.

OMP-94 (Iselin 9402) 'Biology' Spring cruise. Nutrients (at sea), oxygen. A complete duplicate set of nutrient samples was also frozen for subsequent shore-based analyses. Three highly-detailed transects were sampled. Samples were also collected around the OMP moorings. In addition, samples were collected from the northernmost transect (36.5 °N) for shore-based measurements of CT and alkalinity.

OMP-94 (Iselin 94xx) Alongshore 'chemistry' cruise. CT, total alkalinity, oxygen. ~8 moderate- to-high resolution transects were occupied along the shelf from Cape Cod to Cape Hatteras. In addition, there was detailed sampling around the OMP mooring array. The BNL team looked after the LDEO underway pCO₂ system.

OMP-94 (Iselin 94xx) 'Biology' Summer cruise. Nutrients (at sea), oxygen. Three highly detailed transects will be sampled. Samples will be collected around the OMP moorings. In addition, samples will be collected from (at minimum) the northernmost transect (36.5 °N) for shore-based measurements of CT and alkalinity.

Keywords: inorganic carbon, oxygen, nutrients, pCO₂

**PRINCIPAL
INVESTIGATOR(S)**

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PROJECT TITLE OPTICAL ASSESSMENT OF LARGE MARINE PARTICLES:
DEVELOPMENT OF AN IMAGING AND ANALYSIS
SYSTEM FOR QUANTIFYING LARGE PARTICLE
DISTRIBUTIONS AND FLUXES

AMOUNT OF FUNDING FY 1993: \$43 K

SUMMARY OF GOALS

The central goal of DOE's Ocean Margin Program (OMP) is to determine whether continental shelves are quantitatively significant in removing carbon dioxide from the atmosphere and isolating it via burial in sediments or exporting it to the open ocean (Program Announcement, 1991). A major component of the OMP will be to measure carbon flux on the shelf and across the shelf to the slope and open ocean. We are developing a video and optical instrument package (LAPS: Large Aggregate Profiling System) and the analytical techniques to precisely measure a wide spectrum of the large aggregate population of particles in the shelf/slope environment. This particle population, encompassing the "marine snow" size particles (diameters > 0.5 mm), is thought to be the major pathway of material flux in the ocean (McCave, 1975; Asper, 1987; Walsh and Gardner, 1992). Our goal is to use aggregate abundance and size spectrum data along with the CTD, beam attenuation and fluorescence data collected with our instrument package to collect data rapidly, repeatedly and accurately such that it is both linkable to carbon flux and usable in biophysical models. Additionally, measurements of particle flux will be made with sediment traps deployed on the continental slope in conjunction with the physical oceanography program. The combination of profiles and sections of aggregate data along with the measured mass flux and chemistry from the sediment traps will allow for a robust estimate of the mass transport and flux of organic carbon via the aggregate pathway.

SPATIAL AND TEMPORAL SAMPLING SCALES

The LAPS can measure aggregate abundance with high spatial and temporal resolution. The camera/strobe settings are adjustable and can be set to accommodate deep casts or shallow casts. Generally, images will be acquired at a rate of 1 per meter on shallow casts, and 1 per 3 to 5 meters on deep casts (>500 m). The length of time of the cast is dependent on the depth and

the lowering rate. For casts < 400 meters an hour of ship time is required. Deeper casts are proportionally longer with >3000 m casts requiring 4 to 5 hours of ship time. The LAPS package has a pinger and can approach within 5 m of the bottom. Multiple casts can be performed within short (e.g. 24 hour) time periods to acquire data on diel variability.

We envision sampling on cross-shelf and slope lines in conjunction with the CTD, chemistry and biological sampling, as well as sampling at mooring locations (particularly the two sediment trap moorings). Because the LAPS is autonomous with respect to power requirements and requires no special equipment, LAPS profiles can follow CTD casts on section lines.

METHODS AND PLATFORMS

The LAPS as developed for the OMP is an autonomous system consisting of two major subsystems deployed on a rigid frame that is lowered from a ship using a hydrowire. One subsystem consists of a pair of video camera/strobe/battery systems which are independent of each other. The strobe light output is synchronized to its camera such that the camera records a full second of video (Hi-8, 400 line resolution) within which a single frame is illuminated by the strobe. The strobe light output is collimated and baffled to produce a defined slab of light oriented perpendicular to the camera. One of the cameras will be set to record the low range (>250 μ m) of the particle field while the other camera will record the high end (>1 mm). These parameters can be adjusted to the conditions. The second subsystem consists of a Sea-Bird Seacat CTD mated to a Wet Labs ac-3 meter. This subsystem is used to measure physical parameters (T, S, pressure) as well as beam attenuation and fluorescence. Merging the data sets from the two subsystems is accomplished by time syncing. Any oceanographic research vessel capable of supporting a multidisciplinary research effort is sufficient as a platform for the LAPS.

STRENGTHS AND LIMITATIONS OF PROPOSED RESEARCH

Video based systems for measuring aggregate abundance are necessary because aggregate resuspension ('rebound') occurs at benthic shear stresses significantly lower than required to resuspend sediment, resulting in a decoupling of aggregate transport from traditionally measured optical properties such as beam transmission, as observed on the slope in the Gulf of Mexico where aggregate nepheloid layers are found in the absence of a transmissometer nepheloid layer (Gardner and Walsh, 1990; Walsh and Gardner, 1992). In the shelf/slope environment rebound of aggregates and subsequent downslope transport and settling may be a significant mechanism for export of shelf organic carbon, potentially explaining the discrepancy between benthic oxygen demand on the slope and overlying production (J.J. Walsh et al, 1991; Janhke et al., 1993). The LAPS allows for the measurement of aggregate abundance and size distribution on spatial and temporal scales approaching synopticity (i.e. multiple profiles per day closely spaced along the ship track). This capability is required to assess the along shelf and cross shelf/slope variability of the OMP field area.

The major limitation of the LAPS is the time required for post-deployment processing of the imaging data. Current efforts to streamline the computing steps required (e.g. capture, thresholding, particle counting, data sorting and binning, data compilation) have reduced the overall time significantly. However, the total process still requires four to five hours per cast. On-board, post-cast processing and further refinements to the computer programs will reduce the amount of data that needs to be processed ashore. A further limitation on the LAPS is that profiles made during the day need more extensive image processing to account for the ambient light field. However, given that film based systems cannot produce profiles in an ambient light field at all, the ability to produce profiles under any lighting conditions is significant.

STATUS OF RESEARCH

The LAPS will be tested in the field area during a cruise in June/July 1994. Sediment traps will also be deployed on that cruise to make the first comparisons between measured flux and aggregate abundance in the field area. Efforts to streamline the image processing have resulted in a suite of programs to handle the data from capture to binned data.

Keywords: aggregates, marine snow, particle flux, video, beam attenuation

**PRINCIPAL
INVESTIGATOR(S)**

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PROJECT TITLE

NUMERICAL STUDIES OF BOTTOM BOUNDARY LAYER
PROCESSES ALONG THE CONTINENTAL MARGIN

AMOUNT OF FUNDING FY 1994: \$80 K

SUMMARY OF GOALS

- (1) To study the role of the bottom boundary layer (BBL) on the continental margin in exporting water (contaminants) from the shelf into the ocean interior.
- (2) To measure ocean currents in the OMP area to further our understanding of the water mass budgets and their temporal variability in the region.

SPATIAL AND TEMPORAL SAMPLING SCALES

(The following only considers the observational component of what I would like to do.)

The current observations would be a component of the OMP current meter efforts. A tighter, more coherent moored array than that presented at the OMP meeting at BNL last fall, would seem more appropriate for estimating water mass budgets. Thus I am biased towards the L. Pietrafesa type of mooring distribution. I propose contributing about 20 current meters and associated equipment (releases, floats, radio and light beacons, ...); my particular interest would be on the flow seaward of the shelf break. Depending on the moorings, the meters would be left for about 3 months (shallower moorings where fouling is a problem) to about a year (deeper moorings). Such sampling should be sufficient to resolve seasonal variability, particularly winter and summer conditions.

I would like also to deploy two BBL profilers seaward of the shelf break as part of the current meter work described above. Each profiler consists of two current meters, one in and the other above the BBL with supplemental speed sensors interspersed. The intent of the BBL profilers would be to see if the BBL seaward of the shelf break is indeed quite different from the classical Ekman layer as proposed by MacCready and Rhines, 1993, J. Phys. Oceanogr.,21, 1186-1201) and as predicted by our numerical model.

The CTD data obtained so far in OMP has been unsuitable for resolving the BBL. This is due, I believe, to the research interests of those involved in the CTD program and due to instrumentation problems. To help remedy this problem, I would like to participate actively in future OMP CTD surveys. (I am currently on a WOCE CTD/tracer cruise in the South Atlantic.) In addition to some experience and interest in resolving BBLs with CTDs, I can also contribute two CTDs and a bottom finding pinger. No additional CTD cruises are proposed, only that I help in getting the CTD casts closer to the bottom.

METHODS AND PLATFORMS

For the observational component of the proposed work, I would use the equipment of the Current Meter Facility of Florida State University. Most of the equipment, as noted earlier, is scheduled to be retrieved from the South Atlantic in 1995, and should be available for reuse in the latter half of 1995. I would intend to integrate my current meter work as closely as possible with moorings being set by others. However, if needed, we have the capability of setting autonomous moorings. Note, that the current meters we use are designed to be data loggers for other, external sensors, and could be used as such. (Should we consider fitting them with oxygen sensors?)

I would expect to be on the current meter cruises, and I would like to participate in the CTD station work even if that will require going on additional cruises.

For the numerical modeling component of the work, I propose to continue using the model we now have to help explain what we will observe with current meters and CTDs.

STRENGTHS AND LIMITATIONS OF PROPOSED RESEARCH

The strengths of my proposed work are that (1) it would contribute to a better understanding of the currents and the water mass budget in the OMP area (the current meter work), (2) it would yield a better understanding of what the BBL is like in the OMP area (the two BBL speed profiler deployments and my participation in the CTD data collection and analysis), and (3) it would lead to a better understanding of the dynamics of BBLs over sloping bottoms and the importance of the BBL in the OMP area (the numerical modeling of BBLs).

A possible weakness is that I have proposed using existing current meters where, in some cases, ADCPs might be more desirable.

STATUS OF RESEARCH

The current meter equipment exists, and even allowing for some losses from their present use in the South Atlantic, enough should be available for OMP deployment in the latter half of 1995.

The numerical modeling progress has been slower than anticipated. The model needed to be modified to simulate the more demanding conditions than those it was designed for (the deep ocean bottom boundary layer). We now have it making what we think are reasonable simulations of a BBL in the rigorous conditions of large bottom slopes and large buoyancy (Brunt Vaisaila) frequency found seaward of the shelf break. We are currently examining SEEP CTD BBL data (as noted earlier existing OMP CTD data does not reveal the BBL) for examples of conditions to further simulate and test our model. One paper acknowledging DOE/OMP support was recently accepted by Journal of Marine Research.

Keywords: benthic boundary layer, water mass budgets, hydrography, numerical models

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PROJECT TITLE

SEDIMENT MOTION AND BOTTOM BOUNDARY LAYER
DYNAMICS OVER THE MIDDLE ATLANTIC BIGHT SHELF
AND UPPER SLOPE

AMOUNT OF FUNDING FY 1994: \$229 K

SUMMARY OF GOALS

Our goal is to measure near bed sediment response to currents and waves on the shelf. The flux of suspended sediment at various heights through the bottom boundary layer and the total transport of particulates over the water column are the derived results.

SPATIAL AND TEMPORAL SAMPLING SCALES

The measurements of velocity will be made from 50 cm above bottom to 5 m above bottom at a depth of 20 meters to 120 meters across the shelf. We sample at 2Hz for several months. Deployments can be 2 or 3 months long, limited by fouling of the optical turbidity sensors. Our hope is to extend measurements of flux from the bottom to the surface, close enough in height to obtain 80% coherence between adjacent measurements, perhaps 10 meters apart in the midwater and closer at the bottom and top. Mooring should be close enough that flux estimates are coherent to 80% between moorings. We estimate this to be 10 km in the mid-shelf.

METHODS AND PLATFORMS

Optical backscatter measurements of turbidity will be correlated to instantaneous vector velocities to obtain flux of particulates. This in turn will be integrated to yield transport. The platform will be a mooring for the upper water column and a tripod for the bottom portion.

STRENGTHS AND LIMITATIONS OF PROPOSED RESEARCH

The strength of this approach is the direct, *in situ* measurement of flow field and turbidity field over storm cycles and seasonal variations. The limitation is that optical turbidity is not a unique measure of suspended particulate mass, nor is it a unique measure of particulate organic carbon, either of which would be preferred. A calibration relating optical signal to concentration of particles, organic carbon, etc., must be relied upon, and this calibration depends on size distribution and material composition, qualities that change with season and depth.

STATUS OF RESEARCH

Under DOE support in the Ocean Margins Program, Churchill, Williams, and Pietrafesa have deployed a BASS tripod off Beaufort Inlet to measure response of the seabed to storms. This tripod contained five acoustic current meters, six optical backscatter sensors, pressure, and attitude sensors. Ten days of data were taken, including a period of sediment discharge from coastal rivers following a heavy rain and a high wind event. These data are being analyzed.

A second BASS tripod is being prepared for deployment along with the NC State tripod in July 94 off Duck, NC. The two tripods will be recovered in August, redeployed in September, and recovered in December.

Keywords: benthic boundary layer, suspended sediments, turbidity, sediment transport

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PROJECT TITLE **MOORED SENSING SYSTEMS**

AMOUNT OF FUNDING **FY 1994: \$147 K**

SUMMARY OF GOALS

The goal of this project is to use moored measurements of dissolved oxygen to investigate the carbon cycle on the continental margin near Cape Hatteras. The scientific objectives of this project are:

- 1) To quantify the reduction of carbon by photosynthesis using dissolved oxygen measurements.
- 2) To quantify the oxidation of organic carbon occurring below the pycnocline using dissolved oxygen measurements.
- 3) To estimate the air-sea exchange of both CO₂ and O₂ using boundary-layer transport models driven by a limited set of meteorological, pCO₂, O₂ and total gas tension measurements.

To accomplish these goals we propose to deploy oxygen sensors, BNL fluorometers, Fast Repetition Rate (FRR) fluorometers, irradiance sensors, transmissometers, gas tension devices, and a surface buoy to measure delta pCO₂ in the 1996 OMP field experiment. This project includes: instrument preparation, participation in mooring cruises, and data analysis.

SPATIAL AND TEMPORAL SAMPLING SCALES

A three-dimensional moored array of ~16 moorings containing ~100 oxygen sensors is proposed to derive seasonal oxygen budgets from direct measurements. The scales of the moored array are as follows:

Size of Moored Array:	40 km x 40 km
Horizontal Scale:	5-15 km between moorings
Vertical Scale:	5-10 m between instruments
Length of Deployment:	1 year total, 3 deployments
Sampling interval:	~10 minutes

A small shipboard effort will be required to conduct process studies within the moored array. The shipboard spatial sampling scales will be 1 km in the horizontal and 1 m in the vertical.

METHODS AND PLATFORMS

Platforms: Moorings are the primary platform required by this project. A total of 16 moorings are proposed. Several moorings will be enhanced with ancillary sensors to study photosynthesis, biophysical interactions, and air-sea exchange of gases. Mooring cruises will be required to deploy and recover instrumentation.

Methods: Dissolved oxygen measurements can be used to study the carbon cycle because many of the biogeochemical processes that alter dissolved carbon dioxide stoichiometrically alter dissolved oxygen. The stoichiometry of photosynthetic carbon fixation is rigid: one mole of O_2 is produced for each mole of CO_2 reduced. Similarly, the biological oxidation of organic carbon, $(CH_2O)_n + O_2 = CO_2 + H_2O$, consumes dissolved O_2 and produces dissolved CO_2 . The OMP must rely on moored measurements of dissolved oxygen to describe the in situ oxidation and reduction of carbon in the water column because there are no sensors capable of making moored measurements of dissolved CO_2 .

An oxygen array will be designed to measure community metabolism. Current measurements and measurements of horizontal gradients across the array will be used to partition the local O_2 changes into biological and physical components. The biological change at night will be defined as community respiration and biological change during the day will be defined as community production-community respiration. This simple approach will occasionally fail near the surface unless the array is instrumented to provide good estimates of the air-sea exchange of gases.

Models of the Liss-Merlivat genre will be applied to estimate the air-sea gas transfer rate as a function of wind speed and sea state. More process-oriented models will be used if the array is instrumented to measure physical processes in the marine boundary layer. The local air-sea gas exchange will be estimated using the measured concentrations of gas across the interface and the estimated transfer rates.

Photosynthesis will be studied as a process. FRR fluorometers will be deployed on several moorings to independently estimate photosynthesis from stimulated fluorescence. Others projects will use shipboard FRR fluorometers to study the control of photosynthesis and contribute to the analysis of the moored FRR fluorometer data.

STRENGTHS AND LIMITATIONS OF PROPOSED RESEARCH

The carbon cycle on continental margins is difficult to describe from measurements because many of the contributing processes vary rapidly in time and space. Furthermore, the carbon pools that make up the carbon cycle, DIC, POC and DOC, and their rates of change can be measured only from ships. The new capability to make moored measurements of dissolved oxygen allows time series observations of the change in O_2 to be used as a surrogate for changes in CO_2 . Moored measurements of dissolved O_2 can capture episodic events in the carbon cycle. Oxygen is an integrating variable and the measurements from the moored array can hopefully be extrapolated to a larger region.

Oxygen is not a perfect surrogate for CO_2 . Oxygen exchanges much more rapidly with the atmosphere than does CO_2 and the relative exchange rates vary with conditions. The air-sea exchange of CO_2 is best estimated from direct measurements of the difference in partial pressure of CO_2 across the air-sea interface, delta pCO_2 , and physical state of the marine boundary layer. Oxygen budgets for the upper mixed layer must include air-sea exchange of O_2 , which can be estimated from measurements of O_2 and total gas tension.

STATUS OF RESEARCH

Our success at making moored measurements of dissolved oxygen in the SEEP II experiment created considerable scientific interest because such measurements can be used to verify air-sea gas flux models and to create oxygen budgets for the mixed layer. In phase one of OMP, we participated in several field experiments to test instruments and demonstrate that moored measurements of O_2 can be used to study the carbon cycle.

In February 1993, two BNL moored fluorometers, a solar irradiance sensor, and four YSI oxygen monitoring systems were deployed offshore of La Perouse Bank in the North Pacific Ocean (48.5 °N and 127 °W). This heavily instrumented, moored array also included: gas tension devices, acoustic current meters, neutrally buoyant mixed layer floats, several sonar systems, thermistor chains, and surface following platforms. The experiment was designed to measure (1) near-surface circulation, (2) air-sea gas flux and breaking wave dynamics, (3) ocean surface acoustical reverberation, and (4) the near-surface oxygen budget. Results from this experiment are being used to develop measurement strategies for OMP field experiments.

High resolution TOYO data collected in May 1993 aboard the RV Gyre have been used to design a moored array capable of resolving diel changes in O_2 to a specified error. The temperature, fluorescence, salinity, oxygen and photosynthetic parameter fields were analyzed using krigging techniques to derive objective error estimates for proposed mooring designs.

In March 1994, an array of four moorings containing one FRR photosynthesis, eight oxygen, and seven fluorescence sensors was deployed near Cape Hatteras to test our ability to

measure primary production using fluorescence, and to measure the production and consumption of dissolved oxygen. The moorings are scheduled to be recovered in July 1994. The primary objective of this effort is to develop moored measurement techniques to close the oxygen budget on continental margins.

It is technically possible to make moored measurements of dissolved O₂ over periods of 2-4 months that can be used to investigate the carbon cycle.

Keywords: oxygen, photosynthesis, carbon dioxide, fluorometers, moorings, air-sea gas exchange

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PROJECT TITLE

PHYTOPLANKTON EXCRETION REVISITED: HEALTHY
CELLS MAY NOT DO IT, BUT HOW MANY CELLS ARE
HEALTHY?

AMOUNT OF FUNDING FY 1994: \$99 K

SUMMARY OF GOALS

The primary purpose of this research is to develop molecular tools for determining the health of marine phytoplankton on an individual cell basis. Since the definition of *healthy* in phytoplankton cells elusive, we are developing markers for several different metabolic process indicative of physiological state: photosynthetic activity, esterase activity, accumulation of storage products (ie. lipids), and membrane potential. One underlying motivation is to develop methods which will allow us to evaluate the hypothesis that, while healthy cells release very little DOC, many phytoplankton communities are comprised of *unhealthy* or physiologically stressed cells which release a large proportion of total photosynthate into the pool of labile DOC. Another motivation is the goal of determining the growth state of bloom-forming phytoplankton cells when they are observed in samples from the water column or sediment traps.

During the OMP field year, we are particularly interested in 1) identifying and enumerating *net* phytoplankton in the study area; 2) evaluating their contribution to total biomass; and 3) using the molecular probes we are developing to evaluate the role(s) these large cells play in material and energy flux on the continental margin.

In this work, the physiological state of individual cells is emphasized. It differs from more traditional approaches based on bulk measurements. The traditional approach effectively hides any differences in the relative contribution of different taxa or individuals to overall productivity even though most flux processes are sensitive to physiological and taxonomically-determined differences among members of the community.

SPATIAL AND TEMPORAL SAMPLING SCALES

This approach is relevant to, and compatible with, the study of material and energy flux in marine ecosystems on a variety of time and spatial scales. Discrete samples (net tows, bottle

casts, and hydrographic data) must be taken with sufficient resolution to address the specific objectives of the field program. Based on our preliminary data from July, 1993, phytoplankton community structure and chlorophyll concentration are fairly conservative properties (relative to temperature and salinity) on the continental shelf off Cape Hatteras in the summer. In July, 1993, 16 stations positioned five minutes apart in a hydrographic grid on the mid-shelf region off Cape Hatteras (between 36.0 and 36.5 °N, and 75.02 and 75.17 °W) revealed a large, chlorophyll-rich pool of very cold (7-9 °C) water approximately 15 meters thick lying over the bottom of the shelf. Given that the average shelf depth in this grid was 32 meters (s.d. 2.8m), this could be interpreted as a benthic boundary layer occupying roughly half the water column. However, the phytoplankton present in this water mass were planktonic diatoms, primarily from the genera *Leptocylindrus*, *Corethron*, and *Pseudonitzschia*. Data collected using molecular probes suggests that these cells were *healthy*, ie. in a physiological state associated with balanced growth rather than senescence. This is not improbable given the fact that the Secchi depth on the shelf was approximately 18m; assuming a 1% light level 2.5 times the Secchi depth, the entire water column, and the sediment surface, was well within the euphotic zone.

METHODS AND PLATFORMS

None

STRENGTHS AND LIMITATIONS OF PROPOSED RESEARCH

This approach has the advantage of providing information on both the average character of phytoplankton populations, as well as the variance of individual cells. Further, in cases where the populations of interest are bloom-forming diatoms, the data collected using molecular probes combined with chlorophyll fluorescence allow us to determine whether or not the population is in exponential growth, nearing stationary phase, or highly senescent. The main weakness of our approach has been the need to use fresh material, (this problem has been solved for most kinds of samples), and the time it takes to analyse samples by microscopy and image analysis. The latter problem can be solved for many types of samples using flow cytometry.

STATUS OF RESEARCH

The methods needed to process samples for the OMP field year are nearly all in place. This includes chlorophyll size-fractionation, phytoplankton enumeration and staining with lipid probes and esterase activity probes. The membrane potential probe is still under development, as are additional methods for working with preserved samples and flow cytometry. The highest priority for this project is completing the analysis of the data from our July, 1993 cruise, particularly the cross-shelf grid. If the preliminary evidence that the chlorophyll-containing *cold*

pool water observed on the shelf is exported off the shelf holds up, it is a result that should be investigated more thoroughly during the 1995 field year.

Keywords: net phytoplankton, chlorophyll, physiological state, molecular probes

OCEAN MARGINS PROGRAM

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BENTHIC BOUNDARY LAYER

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Aller	Jahnke
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Baskaran	Mayer
Benninger	Pietrafesa
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OCEAN MARGINS PROGRAM

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BNL (8)
SUNY (4)
Texas A&M (4)
SkIO (3)
USM (3)
URI (1)
OrSU (3)
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OCEAN MARGINS PROGRAM
Phase I: Research Summaries